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A PATHOLOGICAL FEATURE OF FLEA-BEETLE INJURY OF POTATO TUBERS[1]

By H. G. MACMILLAN, *Senior Pathologist*, and L. A. SCHAAL, *Assistant Pathologist, Office of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The potato flea beetle (*Epitrix cucumeris* Harr.) (*5*)[2] has long infested potatoes in northern Colorado. Since 1904 the annual loss caused by the insect in that section has usually amounted to $100,000 or more (*2*). The damage caused by the feeding of the adult beetles on the foliage is considerable, but the losses caused by the feeding of the larvae on the tubers are unquestionably of more importance. Under sales competition and grading rules the potatoes suffer, with losses to grower and dealer and to the reputation of the district generally. From year to year the damage has varied in severity. Efforts to control the beetle have been only partially successful. A study of the beetle damage to tubers grown in pathological experiments led to the belief that some additional factors might be responsible for some of the severe injury usually attributed to flea beetles.

REVIEW OF LITERATURE

The literature dealing with the flea beetle is extensive and voluminous. No effort has been made to review it all, as studies of morphology and life history are of no concern here. The statements concerning damage to the tubers are brief and consistent. Stewart (*13*) stated that the larvae made "pimples" on the tuber surface and developed "slivers" in the flesh. Johannsen (*3*), Webster (*14*), and Britton (*1*) reported the same type of injury. None of them, however, mention long tuber scars or worm tracks. Recently Hoerner and Gillette (*2*), in a competent life-history study, mentioned the worm track but briefly.

EXPERIMENTAL FIELD CONDITIONS

At the Colorado Potato Experiment Station, Greeley, Colo., potato diseases are being studied. For some years the flea beetle has been a growing evil, damaging vines and tubers and spoiling the experimental results. In an effort to combat the beetle, all experimental plots, regardless of the purpose for which they were intended, were sprayed with a 5–5–50 Bordeaux mixture to which was added 2 pounds of Paris green per 100 gallons. In times past the beneficial results of

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[2] Reference is made by number (italic) to "Literature cited," p. 814.

this treatment have varied. In 1928 the field was sprayed only twice because a hail on July 19 stripped the vines of leaves, broke the stems and branches, and practically ruined the field for its original purpose. After that date spraying was not considered worth while. By that time, however, the beetles should have laid their eggs (*2*).

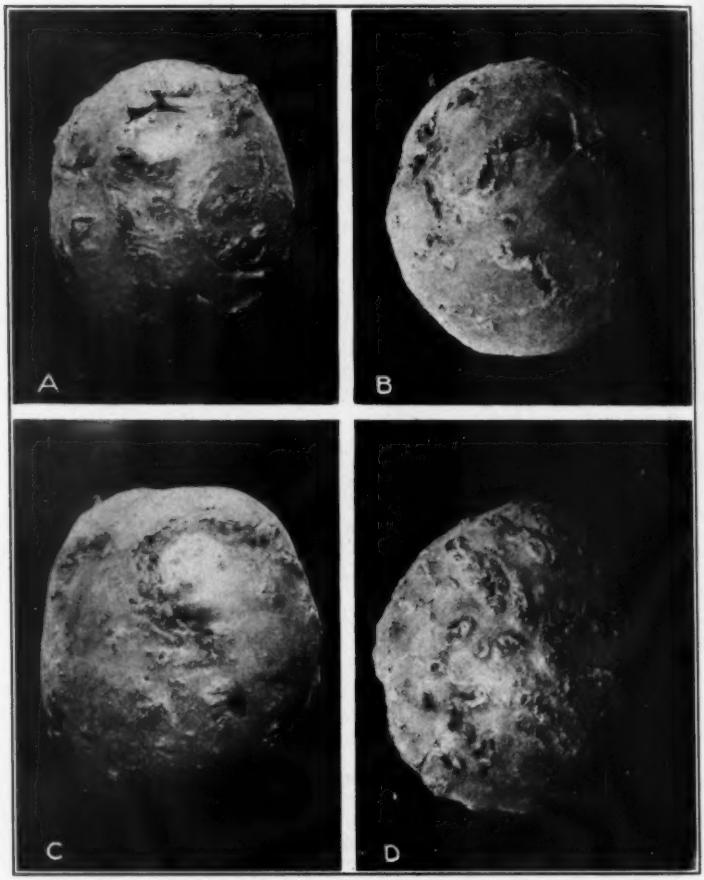

FIGURE 1.—A–C, Tubers showing slight scab-infected worm-track and pimple injuries. The seed potato from which A was grown was treated with graphite, a substance nonpoisonous to pathogenic organisms. The cut seed potato from which B was grown, and the whole seed potato from which C was grown, were untreated. The worm tracks in B were infected with both Actinomyces and Rhizoctonia. D, Scab-infected tuber grown from an untreated whole parent tuber and showing typical pimple development

The experimental field was originally intended to be used for testing seed treatments to control Fusarium blight. The seed potatoes were treated in different ways. After cutting, parts of the seeds were dipped in each of three organic-mercury compounds; and, prior to cutting, other seeds were soaked one and one-half hours in 1:1,000

mercuric chloride. Cut seed dusted with graphite, untreated whole seed, and untreated cut seed as controls completed the experiment. All the seed potatoes were of the Bliss Triumph variety from a single source.

During the season the field was irrigated but twice. Ordinarily five or six irrigations would have been given. Unusually severe

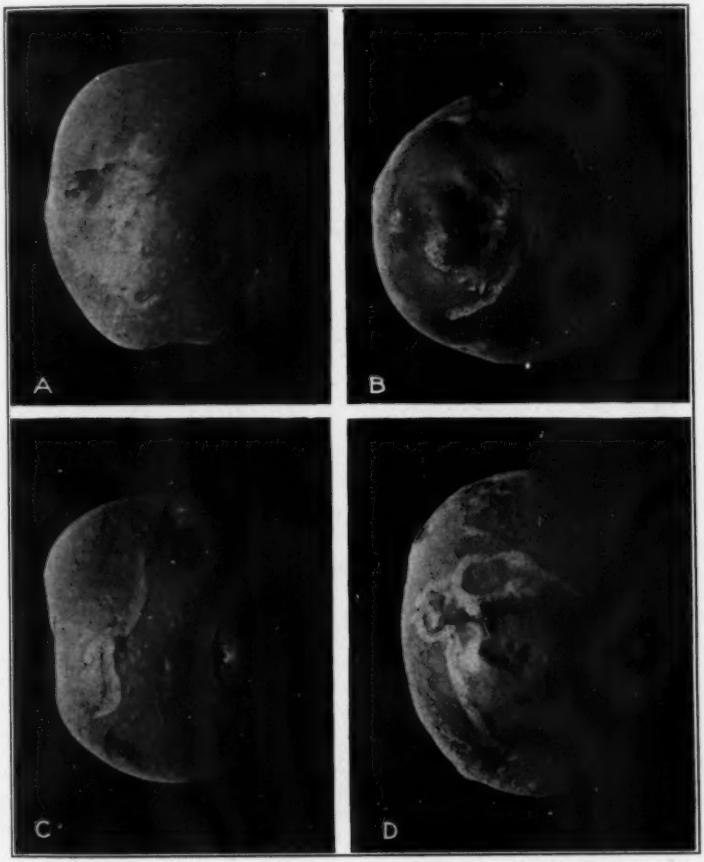

FIGURE 2.—Tubers grown from treated seeds: A, Showing inconspicuous flea-beetle injury; B–D, showing slight pimple and worm-track injury. A seeds were treated with organic mercury *A*; B, with organic mercury *B*; C, with organic mercury *C*; and D, with mercuric chloride

recurring rains made complete experimental management of the field impossible and interrupted the irrigation program.

Throughout the growing season the flea beetles were active and moderately abundant. The infestation was uniform. The foliage injury, as frequent investigation showed, was uniform over treated and untreated lots. The repellent spray was needed early in the growing season. The beetles were to be found after the above-mentioned hail, however.

The potatoes were harvested on September 26 and 27. In sorting, the immediately striking result was the variation in the amount of common scab (*Actinomyces scabies* (Thax.) Gues.) found in the tubers from seed potatoes receiving different treatments. Throughout the field the damaging amount of worm track occurred uniformly where the worst scab appeared. Representative samples from each treatment were reserved for laboratory study.

LABORATORY RESULTS

Of the bushel or more of potatoes saved from each treatment, each of which was a composite sample, all were examined for insect injury. There was some variation in severity in each of the seven lots. From each were selected several tubers characteristic of the lot. They were neither the cleanest nor those with the worst worm tracks. They were entirely average, characteristic, and representative.

The more severely affected tubers were marred by scars, which were often winding and crossing, usually raised, bulging, eruptive, sometimes ravinelike, and discolored in the depths. In addition there were pimplelike swellings that seemed to be bursting from within. The tuber surfaces were rough, eczematous, and decidedly objectionable. The tubers showing the best appearance were smooth. The worm-track scars, though winding like the others, were smooth and only slightly different in color from the normal epidermis. The points at which the larvae had burrowed showed slight scars but no pimples. The older tracks were shallow and narrow, the normal epidermis developing a light feathery edge along the margin. Occasionally there would be a rougher spot, small in extent. The number of worm tracks and the burrows or tunnels were no fewer in the lightly infected than in the more severely infected tubers, but the appearance was greatly improved. The tubers in Figures 1 and 2 are typical of the lots represented. Each shows approximately the same degree of injury from attacks of the larvae, but with very considerable differences in the development of scars.

Tubers which were not attacked by flea-beetle larvae but which bore scab lesions showed characteristic symptoms of scab. The scab spots, which were usually flat, were much larger than a pimple injury, and the interiors were filled with soft corky tissue. True scab injury would not be confused with that caused by the scab organism invading larvae injuries.

CULTURE OF TUBERS

Tubers from each lot were cultured, pieces of tissue being taken from the worm-track areas and pimples. The method described by Shapovalov (*12*) was used. After the surface had been sterilized with hydrogen peroxide, the material was carried to sterile water blanks, and from these to melted nutrient agar, and poured into Petri dishes. In the case of the cleaner tubers, where the worm-track corky tissue was too shallow to be separated, the worm-track tissue was macerated and cultured. The dishes were incubated at 32° C. for 24 hours, examined, and isolations made. At a later time the growths were identified. The result of the culture work is shown in Table 1.

TABLE 1.—*Results of culturing representative worm-track and pimple injuries on tubers grown from treated and untreated seed*

Treatment of seed	Tubers cultured	Tubers yielding Actinomyces scabies		Tubers yielding Corticum vagum	
	Number	*Number*	*Per cent*	*Number*	*Per cent*
Untreated:					
Cut	32	26	81.25	8	25.00
Whole	32	22	68.75	3	9.37
Graphite	20	18	90.00	3	15.00
Total or average	84	66	78.57	14	16.66
Treated:					
Organic mercury A	29	5	17.24	1	3.44
Organic mercury B	30	5	16.66	0	0
Organic mercury C	23	3	13.04	0	0
Mercuric chloride	27	5	18.51	0	0
Total or average	109	18	16.51	1	.92

DISCUSSION

The tubers of the untreated group show 78.57 per cent scab infection in the worm-track areas as compared with 16.51 per cent infection in the treated group. These results are not strictly comparable, however. In the untreated group the worm-track areas were heavily scabbed; the surface corky tissue was lifted off, and the tissue to be cultured was taken from beneath. In the treated group a thick corky tissue had not developed, and the worm-track areas were macerated and cultured. In examinations of the plates from the treated group the scab organism was commonly found growing from the epidermal surface and not from the corky tissue beneath. It appeared to be a surface-contaminating organism and not an infecting one. While the percentage (16.66) of Rhizoctonia found in the untreated group is moderate, the damage resulting from it was small. The worm tracks were damaged mostly by scab.

TABLE 2.—*Total and nonmarketable (scabbed) yields of potatoes, graded to marketable size, grown from treated and untreated seed*

Treatment of seed	Units[1]	Total weight	Scabbed potatoes	
			Weight	Per cent
Untreated:	*Number*	*Pounds*	*Pounds*	
Cut	27	7,397	965	13.04
Whole	9	2,386	302	12.65
Graphite	9	2,288	249	10.88
Total or average	45	12,071	1,516	12.56
Treated:				
Organic mercury A	9	2,450	40	1.63
Organic mercury B	9	2,535	38	1.49
Organic mercury C	9	2,168	40	1.84
Mercuric chloride	9	2,517	63	2.50
Total or average	36	9,670	181	1.87

[1] Each unit consisted of the total yield of a portion of row 300 feet long.

The weight and the percentage of scabbed potatoes found in the experimental field are shown in Table 2. The potatoes were sorted

for marketable size, and those that were nonsalable because of scab were taken out. This classification did not necessitate the elimination of tubers damaged by clean or scab-infested worm track. Table 2 shows that of the tubers grown from untreated seed 12.56 per cent (an unusually high percentage for the Greeley district) were unmarketable because of scab. Of the tubers grown from treated seed only 1.87 per cent were scabbed. It appears therefore, that the treatments, besides reducing infection of worm tracks, decrease scab.

The infection of the worm-track areas of the tuber by *Actinomyces scabies* and by the Rhizoctonia fungus (*Corticum vagum* Berk. and Curt.) has been observed for some time. Figure 3 shows a tuber of the Pearl variety deeply corroded by Rhizoctonia following worm-track injury. In the Greeley area scab and Rhizoctonia are usually moderate parasites, requiring suitable weather conditions for their best development. In 1920, at the place where the tuber shown in Figure 3 was taken, Rhizoctonia was well developed on all tubers where injuries to the skin rendered infection easy. Scab likewise attacks tubers where the epidermis is thin, for thickness of the epidermis is one of the essential natural scab-resisting features of the potato. Jones (*4*) early noted that Cambridge Russet was scab resistant. Lutman (*7, p. 30*) found that the thickness of the skin determines the resistance of the tubers to scab. The effect of the feeding of the larvae on the tuber surface is essentially to reduce the thickness of the periderm. The larvae eat away the epidermis and a few layers of cork cells beneath. More cork cells are produced from the phellogen to repair the damage, but a thin place remains in the protective covering of the tuber. Figure 4 shows a free-hand section through an uninfected worm track. At *a* is shown the normal epidermal tissue, consisting of the thickened epidermis, beneath which are the compact cork cells, and at *b* is the worm track itself, without epidermis but showing the growing cork cells. It is in this tissue that infection occurs. Lutman and Cunningham (*8*) found that the loose cellular formation of the tuber lenticel normally offered easy entrance to the scab organism. Lutman (*6*) had previously found that scab frequently occurred at lenticels. Recently Sanford (*11*) stated that lenticels are the principal points of scab infection. The injury caused by the larvae of the flea beetle creates a similar point of entry for the scab organism.

FIGURE 3.—Pearl tuber grown from untreated seed; badly damaged by Rhizoctonia which followed flea-beetle injury

The conditions that favor the development of scab in the Greeley area differ from those generally reported as favoring scab develop-

ment. Sanford (*10*) found that abundant scab developed in very dry soils, while soils sufficiently moist produced almost clean tubers. Millard (*9*), commenting on the occurrence of scab in England, stated that it is more prevalent in dry than in wet seasons. Other investigators have contributed similar evidence. In the Greeley area the greatest scab development is during wet years, or on wet ground. The growing season of 1928 was unusually wet. Between June 15 and August 1, 6.53 inches of rain fell at the experimental field, or over one-half the average annual precipitation. The amount of scab aside from worm track was much larger than usual. In dry years scab is commonly found only at the lower ends of rows where irrigation water remains longest and drainage is poorest. Hoerner

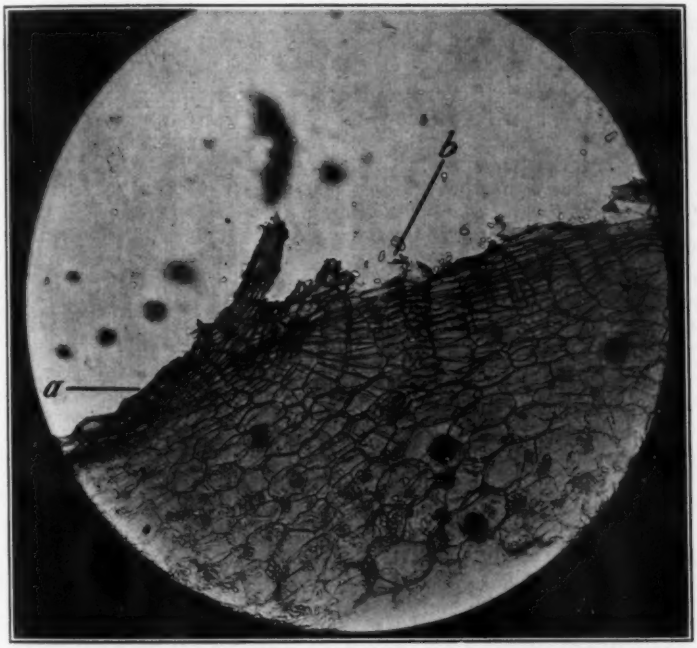

FIGURE 4.—Photomicrograph of a free-hand section through an uninfected worm track, showing normal epidermis and cork cells at *a*, and a portion of epidermis fed upon by a larva that caused the worm-track scar at *b*. This is without epidermis and shows loose cork cells formed from the phellogen

and Gillette (*2*) found that the severe worm-track injury occurred in wet ground or at the lower ends of irrigated rows, and also that smooth-skinned potatoes showed the greatest injury. The writers have made similar observations in regard to scab development in the Greeley area.

A suitable seed treatment effectively controls common scab under the conditions prevailing at Greeley. By eliminating the possibility of infection of the worm-tracked or tunneled areas, the most obvious damage to the tuber may be greatly reduced. The worm-tracked areas are relatively inconspicuous, and the points at which the larvae

tunnel do not develop the pimple type of injury. The question may well be asked whether the pimples described by entomologists (*1*, *3*, *13*) were not infected by scab. Where Rhizoctonia has been the active parasite following worm track the injuries are much more severe. Suitable treatment of seed potatoes for the control of scab and Rhizoctonia is an essential factor in the reduction of the tuber injury caused by the larvae of the flea beetle.

SUMMARY

The flea beetle (*Epitrix cucumeris*) has long damaged potatoes in the Greeley area of Colorado. The foliage is eaten by the adult beetles, and the damage caused by the larvae when feeding on the growing tubers has been a direct cause of economic loss. No complete control of the beetle or its larva has been found.

The damage caused by the larvae is of two kinds. The tuber surface is marred by the so-called worm tracks, and the places at which the larvae burrow into the flesh develop pimples. In the tuber flesh beneath the pimples are cores or slivers, which are found when the tuber is pared. The feeding of the larvae on the tuber, either in the portion appearing as tracks or at the point of burrowing, makes thin spots in the periderm, favorable to the entrance and infection of the spot by the scab organism (*Actinomyces scabies*).

If the seed potatoes have been suitably treated with mercury compounds the scab organism usually does not infect the larvae-injured potatoes. The tubers from treated seed, while attacked by the larvae as severely as tubers grown from untreated seed, show relatively little surface eruption or pimples. They are clean, bright, and smooth.

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OBSERVATIONS ON TASSELS OF TEOSINTE MALFORMED BY SCLEROSPORA [1]

By WILLIAM H. WESTON, jr., *Professor of Botany, Harvard University,* formerly *Pathologist, Office of Cereal Crops and Diseases,* and J. H. CRAIGIE, *Plant Pathologist, Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada,* formerly *Agent, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.*[2]

INTRODUCTION

One of the most striking and commonly reported effects on cereals and other gramineous hosts resulting from attack by Sclerospora is the malformation of the inflorescence. This feature usually has been emphasized as conspicuous by many investigators during the last 50 years, who described these diseases on an extensive list of hosts, both cultivated and wild, comprising representatives in all but one or two of the less important tribes of the Gramineae. Of the tribe Maydeae (Tripsaceae), maize (*Zea mays*) has been found in Java, India, Formosa, and the Philippines to be seriously attacked by five separate species of conidial Sclerospora (*6, 35, 38, 45, 46, 54, 56*),[3] at times with some concomitant malformation of the inflorescence. Yet in the case of teosinte (*Euchlaena* spp.), although it is perhaps the closest relative of maize and (as might be expected therefore) also is susceptible to these downy mildews, no instances of deformation have been reported hitherto. In the Philippines, however, while studying the Sclerosporas chiefly destructive to maize but also attacking teosinte, the senior writer, during two years of investigation and among many teosinte plants relatively unaltered by such attack, did encounter certain cases of abnormalities developing as a result of inoculation with *Sclerospora philippinensis*. It is with the hope that their rarity, their remarkable structure, and their possible significance may render them of interest that these cases are described in the present paper.

HISTORY OF THE CASES

The first planting of teosinte at the College of Agriculture of the Philippines was a small experimental plot started in July, 1917, with seed of *Euchlaena luxurians* Schrad. obtained from a New York seedsman. With seed from this a second planting was grown from June to November, 1918. In this second plot, and among the volunteer plants which sprang up around the maize then planted in the first, many instances of infection by *Sclerospora philippinensis* were noticed, but only a few were at all deformed and these but slightly (*54, p. 104*). With healthy seed from this plot seedlings were grown in pots protected in the laboratory. Beginning January 25, 1919, when they were 9 days old, with two leaves and about 3 inches high, the seedlings were placed for seven successive nights under young

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[2] To G. N. Collins for his kindness in critically reading this manuscript the writers wish to acknowledge their indebtedness; also, to the Milton Research Fund of Harvard University the senior writer wishes to acknowledge his gratitude for aid which, by lessening some of the demands of class work, has expedited the long-delayed preparation of parts of this paper.
[3] Reference is made by number (italic) to "Literature cited," p. 833.

maize plants infected with *S. philippinensis* and showing abundant nocturnal production of conidia. To prevent extraneous infection the seedlings were covered each night with large cans. After being thus subjected to certain infection, they were allowed to develop in the laboratory until, on February 6, several of them (now 5 inches high and with the third leaf developing) showed the first paling leaf areas that precede production of conidia (*57*). Sixty of these seedlings were then planted in a corner of an isolated abaca and tobacco patch far from any other cases of downy mildew, and the progress of the disease on individual plants and in the plot as a whole was followed.

By the end of March many plants had died, under these crowded conditions, leaving only nine living, of which three were still healthy and remained so. In late April and early May, 1919, when conidiophore production on the six diseased plants had ceased save on young leaves of shoots recently put out, the male inflorescences (tassels) both on the main axes and on side shoots were found to be developing into abnormal, malformed structures, in contrast to the normal ones of the three still healthy survivors. These tassels, instead of producing pollen and becoming passé, remained green, the deformed glumes and lemmas of the hypertrophied spikelets continuing to grow excessively into contorted, elongate, bractlike structures which gave them a most bizarre appearance. A representative specimen was photographed (fig. 1) and preserved in copper acetate solution for future study; the others were kept under further observation.

During June a few additional tassels, also malformed, continued to develop from the later suckers of the diseased plants. This isolated plot was not visited during July, as in the latter part of that month there began a series of successive and severe typhoons that raged as one intermittent storm for six weeks. When visited on August 6 the plants were found to have been blown over and beaten down into the mud by the violence of the storms. The plants themselves had responded indomitably to this severe treatment by taking root and sending up new shoots at each node, but of the remarkable malformed tassels only a few survived, the several others, already old, having been beaten and soaked to pieces. These few survivors were collected, and from two of them were taken the peculiar rooting plantlets which, as herein described, were planted in the endeavor to foster their independent growth. As these malformed tassels seemed to present features of unusual interest, all possibly pertinent information concerning them was recorded, various illustrative photographs were taken, and the specimens themselves were preserved in formalin and in alcohol for more thorough examination later.

BEHAVIOR OF THE MALFORMED TASSELS

When the few plants whose malformed tassels had survived the severe storms were collected on August 6 it was with the intention of making a complete record of each one by means of photographs and notes, and this was carried out in part. The storms, however, became so violent that they prevented the completion of the work, and two remaining plants were left in the shelter of a tree on the lawn. These showed remarkable behavior, for when they were examined on August 13, during a lull in the storm, the upper portions with only slightly

deformed spikelets were found to have rotted apart and disintegrated, while the strikingly malformed spikelets of the more basal portion of the tassels had remained green and sound. Indeed, several of the

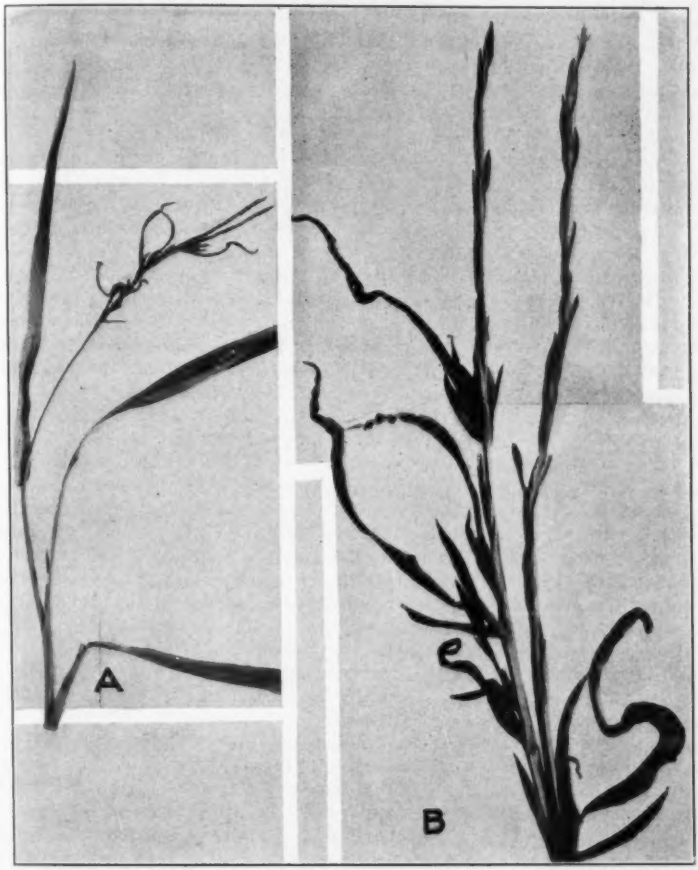

FIGURE 1.—Malformed tassel of teosinte: A, Upper portion of a plant of teosinte infected by *Sclerospora philippinensis*, showing the malformed tassel in position. This plant was one of a lot sprouted from seed planted in pots on January 17, 1919, inoculated as young seedlings with conidia of *S. philippinensis* from maize during the nights of January 25 to 31, planted on February 6 in an isolated plot, where, supporting the disease with little apparent effect beyond the striping of conidiophore-bearing areas of the leaves, the excessive production of tillers, and the suppression of the female inflorescence, the plants in late April developed the tassels. In June, when this specimen was photographed, the infected tassels were still flourishing vigorously, in marked contrast to the already passé tassels of comparable healthy plants. B, The same tassel more highly magnified to show its structure in greater detail. It consists of two long, approximately equal branches bearing spikelets, all of which are sterile, but most of them superficially relatively unchanged, although a few, as the one spikelet at the base of the right-hand branch and the six spikelets in the lower half of the left-hand branch, show malformation into striking, contorted outgrowths involving especially hypertrophy of the glumes and lemmas. The illustration is from two smaller photographs pasted together. About three-quarters natural size

more elaborately hypertrophied spikelets, resembling deformed seedling plantlets, had actually sent out roots from their bases (fig. 2, A, B, C, D) as though they were capable of independent existence; whereas

some, as shown in Figure 2, A, *a*, *c*, and *d*, had practically separated from the prostrate tassel and had changed their orientation with relation to it until they had almost assumed the upright position which would be natural to young seedling plants of their size. Several of these, including those illustrated in Figure 2, B and D, were planted in a pot. Although they remained vigorous and continued to grow for more than a week longer, they were unable (probably in part because in the continuing storms they had almost no sunlight and were constantly far too wet and exposed) to establish themselves as independent plants and ultimately became water-soaked and rotten.

The development of the branchlets of these deformed tassels into entities comprising leafy shoots, giving rise at their bases to roots, orienting themselves like young plants, and for a time continuing to grow when quite separated from the tassel of which they had been a part, seems to justify regarding these as cases of apogamy. This holds, of course, only if the term is used in its general application, that is, to instances in which new plants develop nonsexually from parts that normally produce sexual organs, and not if the term is used in its more restricted cytological application. The behavior of these specimens, therefore, is of some interest, for in the Gramineae cases of apogamy in general are rare, whereas cases resulting from the attack of parasitic fungi have not been reported, as far as the writers are aware. As might perhaps be expected from the close relationship of the two genera, the examples of apogamy in maize (healthy, not parasitized) described by Collins (*9*) show striking resemblance in structure to those of teosinte now under consideration, although it should be noted that the plantlets which developed in the maize tassels terminated in small female inflorescences, whereas those of teosinte, if not completely sterile, showed only male rudiments. Moreover, the plantlets from the maize tassels when transplanted by Collins, even though they did not reach maturity, grew in an apparently normal way for nearly two months, producing roots more than a foot in length, thus far exceeding the amount or extent of the continued growth of the teosinte plantlets. The growth of these teosinte plantlets, however, even though scanty, was enough to show certain interesting aspects, for their development apparently resulted from attack by the Sclerospora, or at least followed it, and if they had lived they would have transmitted a novel type of primary infection to the plants into which they matured, since as long as the plantlets persisted they harbored within their tissue mycelium that remained living and apparently quite capable of renewed growth.

GENERAL STRUCTURE OF THE TASSELS

The appearance of the tassels as a whole was more or less striking, depending on the degree of malformation, but in all cases it was enough to arrest attention. In contrast to the normal graceful tassel with its several slender digitately diverging branches set with regular rows of small, smoothly fusiform, tapered spikelets of appressed florets, the inflorescences were frequently telescoped with reduced branching to bunchy, tufted heads, or rendered unsymmetrical and bizarre in contour by flaring, uncouth, sprawling outgrowths.

In the bunchy type, representing one extreme of the forms encountered, the branching was suppressed, the whole inflorescence being

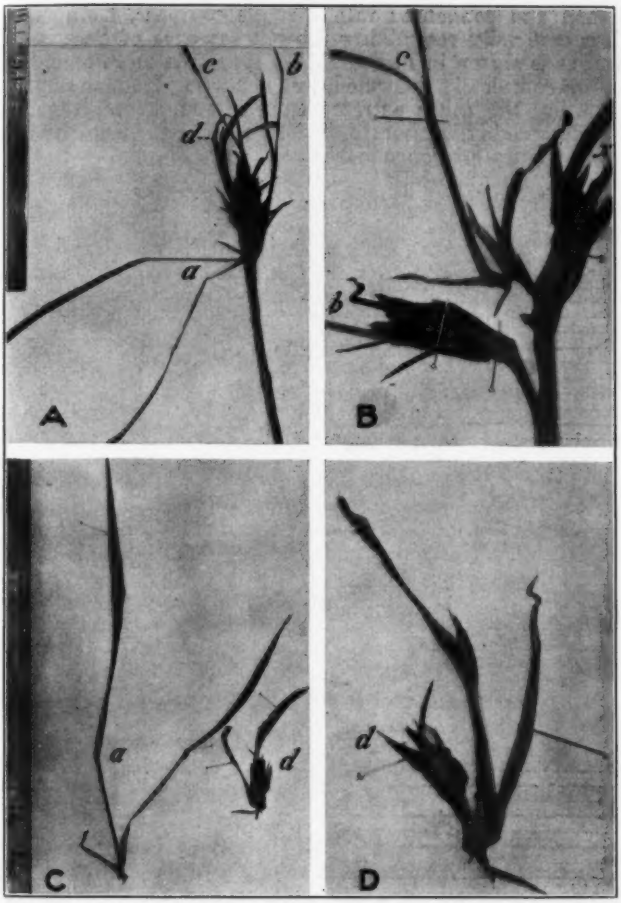

FIGURE 2.—Malformation and root development of parts of teosinte tassel: A, The basal portion of a male inflorescence of teosinte taken from a plant of the same lot and with the same history as the one illustrated in Figure 1. This plant, however, after surviving a long period of incessant storms until August 6, was pulled up and then left lying during an especially violent typhoon until August 13, when, although the top of the tassel had rotted apart and the less malformed spikelets of that portion had begun to disintegrate, the more elaborately malformed spikelets at the base of the inflorescence had continued growing and had sprouted roots at their bases. Scale in inches. B, The base of the same tassel with some of the seedlinglike branchlets removed and the remaining parts separated to show the general structure. The branchlet *c*, previously obscured by the lower clump of spikelets *b* now pinned down away from it, shows a general resemblance to a seedling plant with its base, almost separated from the tassel axis, having one short but obvious root growing down from it. The broken end of the main axis, from which the top of the tassel disarticulated, is shown at *x*. About two-thirds natural size. C, Two of the deformed parts, *a* and *d*, removed from the tassel shown in A. The slender one, *a*, is recognizable as a single spikelet with glumes and lemmas so overgrown into elongate leaflike structures (6 and 8 inches long) that the whole entity with its few short, poorly developed roots at the base resembles a sickly seedling plant of teosinte. The shorter one, *d*, is a compact clump comprising at least two spikelets, much malformed, rooting at the base. Scale in inches. D, The malformed branchlet, *d*, already shown in C after removal from its place (*d*) in the tassel of A, spread apart and photographed somewhat larger to show the general structural features of the several parts and the growth of the roots at their base. About two-thirds natural size

shortened and condensed into a single compact, clumped, bunchy head, superficially resembling the inflorescence of Setaria or Pennisetum, as is shown in Figure 2, A. Even the more open branching type, less completely deformed, less departing from the normal, and representing the other extreme encountered, showed more or less numerous striking, elaborate, twisted, curved outgrowths that, as shown in Figure 1, at once marked them as abnormal. In their gross structure these male inflorescences presented a striking contrast to those healthy ones illustrated and described by Collins (*14, 16*), by Weatherwax (*53*), and by others. While more detailed morphology and histology of these malformations will be considered later, it is worthy of note that in general the spikelets of the tassel, especially the more basal ones (fig. 1), were strikingly hypertrophied, the lemmas particularly enlarging astonishingly into expanded leaflike structures (fig. 2), and the whole spikelet elaborated until in some cases it much resembled a somewhat deformed seedling plantlet (fig. 2, B, C, D) sprouting from the joints of the tassel branches. Moreover, the stamens were lacking entirely or else abortively developed, so that all the spikelets of the tassels were sterile. This was true not only in the badly deformed, more basal spikelets, as might be expected, but also in those usually nearer the tip, which appeared superficially unaltered.

In view of the elaborate development of these deformed spikelets, it is remarkable that all the cases showed no malformation except in the tassels. The plants themselves were not seriously injured and, like most of the Sclerospora-infected teosinte individuals already studied, described, and illustrated by the senior writer (*57, pl. 6; 54, pl. 22c*), had shown during the period of conidiophore production relatively inconspicuous striping and stiffening of the leaves, had continued growing unimpeded, and at maturity, when production had ceased, were only faintly mottled, although the mycelium was still present in the leaf tissue. Moreover, these plants without exception did not develop any female inflorescence, thus lacking opportunity for the malformations that frequently occur in those structures in downy-mildewed maize plants.

DETAILED STRUCTURE OF THE TASSELS

The representative specimens which had been preserved in the Philippines eventually were brought to Harvard University, where a detailed study of the floral structure and the histology of the tassels was made by the junior writer. The floral parts of these malformed specimens were carefully unrolled, dissected, drawn under the camera lucida, and compared with one another and with healthy material. Also, critical structures and parts were embedded in paraffin, cut in several directions in sections of various thicknesses, and stained with various combinations, such as Flemming's triple, Pianese 3, or haematoxylin and Orange G.

As the general symptoms of this disease in teosinte plants have been described in earlier papers by the senior writer (*54, 57*), attention was directed to the staminate inflorescence in which the malformations occurred.

The normal, healthy, staminate inflorescence of teosinte resembles that of Indian corn except that in the former the central spike is suppressed. The spikelets are 2-flowered and arise in pairs at each

joint of the rachis and on one side of it. One spikelet of each pair is subsessile, the other one is pediceled. These features are well known from the descriptions and illustrations of Collins (*13*, *16*), Weatherwax (*53*), and others.

In the tassels of the diseased plants various gradations of transformation existed. As a rule, the sessile spikelets were more excessively malformed than were the pediceled ones, and those at the base of the spike more than those toward the tip. In some of the diseased plants the general appearance of the spikes was not greatly altered, but in other cases only a few joints of the rachis developed, and the spikelets here were generally contorted and excessively hypertrophied, so that they presented a "bunchy" appearance in which practically all semblance of a spike was lost. Each joint of the rachis, however, almost invariably bore its two spikelets. Sometimes both of these were pedicellate.

One case different from any of the others was observed. Two spikelets were raised on a short pedicel and inclosed by three glumes. (Fig. 3, H.) One of these glumes was somewhat larger than either of the other two, but there were no indications that it resulted from a fusion of two. That these spikelets were indeed spikelets and not florets is shown by the fact that each one possessed two florets whose composition and arrangement were identical with those of other diseased spikelets.

Gabotto (*19*) relates that in a study of maize infected with *Sclerospora macrospora* he counted 60 virescent staminate inflorescences of abnormal development and states that, since the plants did not produce ears, the parasite seemed to stimulate excessive growth of some parts at the expense of others. A somewhat similar effect was evident in this material. In those branches which retained their spikelike formation the excessive overgrowth of the spikelets on the lower portion of the rachis seemed to take place at the expense of spikelets higher up. Indeed, from the base to the tip of the rachis there was manifest a tendency toward the progressive reduction, and finally the suppression, of the several parts of the florets. The second floret of each spikelet was invariably smaller than the first floret and first suffered the loss of one or more of its floral elements. At a distance up the rachis of approximately one-third the entire length it lost its palea. Slightly higher a similar loss was sustained by the sessile floret. The lemmas of each of the florets were still present at that height, but about halfway up the rachis the lemma of the second floret usually disappeared, and somewhat farther up the first floret lost its lemma, so that in the topmost spikelets, more often than not, only the two glumes were present. (Fig. 3, F.) Occasionally the first glumes alone remained. As the spikes of diseased tassels differed much in length and were always shorter than those of healthy tassels, it can readily be understood that in extreme cases the upper portion of the spikes would fail to develop at all and the bunchy type of tassel already mentioned would result. In the more detailed account of the floral structure which follows, the features referred to will apply particularly to excessively hypertrophied spikelets, the applicability of the description diminishing proportionately as the spikelets approached normal development.

The glumes shared in the general overdevelopment of the spikelet, but rarely lost their glumelike character. Their increase in width and thickness was proportionate to their increased length, which in many

cases exceeded three times that of the normal glume. (Fig. 3, A, B, H.) More vascular bundles were present than in the healthy glumes,

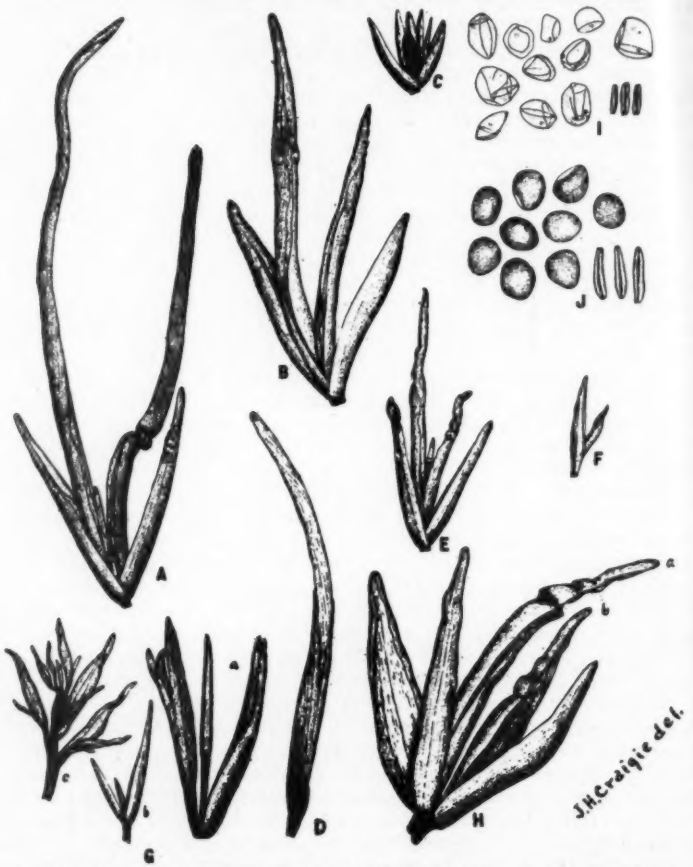

FIGURE 3.—Structure of malformed spikelets from tassels of infected teosinte: A, B, E., Staminate spikelets of teosinte hypertrophied and malformed by an attack of *Sclerospora philippinensis*. The tightly enwrapped floral elements were opened and spread apart to show the position and relative size of each. In A the lemma of the second floret had to be twisted from its original position in order to expose the palea. The portion above the knotlike formation was not enveloped by the other lemma. × 1½. C, Spikelet of healthy tassel, showing stamens in first floret, and affording an interesting comparison in size, proportions, and construction to those of the diseased. × 1½. D, Spikelet of diseased tassel showing hornlike form characteristic of many diseased spikelets. × 1½. F, Spikelet from tip of a diseased tassel, floral parts reduced to two. × 1½. G, *a*, Three spikelets which arose at one joint of a rachis; *b*, central spikelet opened, consisting of three floral parts; *c*, proliferation of florets found within the glumes of the left spikelet of *a*. × 1½. H, Two spikelets, *a* and *b*, growing on a common short pedicel and inclosed by three glumes. × 2. I, Pollen grains and anthers from diseased florets. The grains are shrunken and collapsed, with variously wrinkled and infolded walls and with content almost devoid of protoplasm, so that the germ pores appear very distinct. The anthers from the diseased florets are small and poorly filled out. Pollen grains, × 78; anthers, × 2½. J, Healthy pollen grains and anthers, drawn to the same scales of magnification as I, in order that they may be compared with the pollen grains and anthers of the infected plants

and as a consequence of this and the greater thickness the glumes were quite rigid. The hispidness characteristic of the glumes was much more pronounced in these than in the healthy ones.

The lemmas, on the other hand, underwent astonishing transformations and usually assumed a very extraordinary arrangement. Although in healthy florets the lemmas are membranaceous and hyaline, with any trace of hispidness visible only microscopically, several of the diseased ones were leaflike and virescent, while most of the others were distinctly hispid and rigid, resembling very much in texture the diseased glumes. These observations apply more particularly to the lemma of the first floret of each spikelet, for in most cases the arrangement of the floral parts seemed to forbid such extravagant development in the other parts as was permitted in this one. In practically every diseased spikelet examined, the lemma of the first floret tightly enwrapped its palea (and stamens when present) and the second floret either wholly or in part. In doing this it usually assumed a somewhat spiral course, so that when it completley enveloped the other floral elements it presented the appearance of a horn. (Fig. 3, D.) Occasionally this formation was quite regular, but more frequently it showed various degrees of torsion and wrinkling. (Fig. 1, B; fig. 3, E.) When the second floret was not completely encircled, the upper portion being sometimes uncovered, the exposed portion was very similar in appearance to the enwrapping lemma. (Fig. 3, A.)

In a few cases there were modifications of this general arrangement. The most interesting one of these was the differentiation in several cases of extremely enlarged lemmas into sheath and blade, as in a leaf. (Fig. 2, B.) The lower portion was tightly enrolled after the manner of a sheath, but the upper portion flattened out into a well-formed blade. (Fig. 2, A, B.) Just at the top of the sheath a ligule was almost invariably present. It never developed completely around, but extended inward from either edge for about one-third the width. The central portion of the ligule failed to develop. In the cases just mentioned the upper portion of the lemma of the second florets was left exposed, and two or three of them expanded into virescent blades, but no ligule formation was observed.

Toumey (*50*), in describing abnormalities in the inflorescence of *Phleum pratense*, mentions that some of the flowering glumes were changed into leaves. Butler (*5*) did not observe any transformation of this kind in *Pennisetum typhoideum* attacked by *Sclerospora graminicola*, although other abnormalities were produced. He states, however, that the lemmas were usually elongated, occasionally virescent, and softer than healthy ones.

Although each joint of the rachis regularly bore two spikelets, a case was observed in which three spikelets arose from the same joint (fig. 3, G, *a*), one of which was subsessile, the other two pedicellate. Of these last two, one was situated centrally and possessed but one floret (*b*), the other resembled in external characters the ordinary diseased spikelets, but when its glumes were removed it disclosed a very unique construction. The rachilla, which in other spikelets bore two florets, was elongated considerably and bore seven spikelets. (Fig. 3, G, *c*.) These were not arranged in quite the orderly manner that was evident in the ordinary spike. The first spikelet on this elongated rachilla was sessile, the next one above it pediceled. The fourth was raised on a comparatively long pedicel in the axis of the third, which was sessile. The three uppermost arose separately and were greatly reduced, two of them being represented by only a single glume each, the third one by two very

diminutive glumelike structures. In this particular case the rachilla had evidently assumed the function of a rachis and bore spikelets instead of florets. The term "spikelet" is applied to these structures for the reason that the glumelike character of the outer element of each was very evident, and one spikelet consisted of three bracts, whereas, if it were a floret, it would have had but two—the lemma and the palea.

This aberrancy would seem to fall in teratological classification under "median prolification." Moquin-Tandon (*37*) speaks of prolification as "median" when an adventitious bud springs from the center of the flower as a direct continuation of the growing point. In describing prolification in flowers, Masters (*32*) states that in median prolification the adventitious bud springs from the center of the flower; the usual arrest of growth which occurs at this spot no longer holds good, but a new growth takes place, manifesting itself generally in the formation of a new flower bud, a new leaf bud, a branch, or even in the production of an inflorescence. Toumey (*50*) described a case in which some timothy spikelets had four glumes, some only two, and between the inner ones a long stipe, generally more or less twisted, arose and was surmounted by a perfect flower. This irregularity in structure Penzig (*44*) termed "central prolification."

The dissimilarities of the internal structure of healthy and diseased lemmas were not less marked than those of their outward form. Healthy lemmas, for the most part, were of a uniform thickness, mainly one cell thick, and therefore contained no mesophyll or well-defined vascular bundles. Diseased ones, on the other hand, were of uneven thickness, having well-developed mesophyll and fairly regularly spaced vascular bundles (fig. 4, F, H), some of which were quite perfect, with vessels, phloem, and sheath, while others perhaps, consisted of only two or three vessels, or presented gradations between these two extremes.

As the morphology of those portions which flattened out to form blades was considerably different from that of the rest, it will be mentioned first. A comparison of the two cross sections F and G of Figure 4 shows how closely the former approximated the regular leaf formation of the latter. In both cases the upper and lower external walls of the epidermis were of about the same thickness, although in the leaf, G, these were slightly thicker than in the bladelike lemma, F. Also, the leaf itself was somewhat thicker. The vascular bundles in the bladelike lemma were regularly spaced and were as complete as many of those in the leaf, yet at intervals the leaf possessed bundles which, above and below, were abutted by a group of thick-walled sclerenchymatous cells which extended to the epidermis. These were absent in the bladelike lemmas, but in general their structure corresponded very closely to that of the leaf.

The inrolled lemmas, on the other hand, differed quite materially from the flattened portions or from a leaf. As seen in cross section, their margins were thin, but a progressive thickening took place toward the middle, so that in this region there was a spongy mesophyll, as in the leaf. (Fig. 4, D.) There were present also more or less regularly spaced vascular bundles. Toward the edges, and therefore in the thinner portion, these were always incomplete, being composed usually of a few vessels. In the thicker portions some were quite complete (fig. 4, H) and others were imperfect like

those in the thinner parts. The cell walls of the outer epidermis were thick and sclerenchymatous, and they frequently protruded, so that this surface was studded with short stout hairs. Stomata

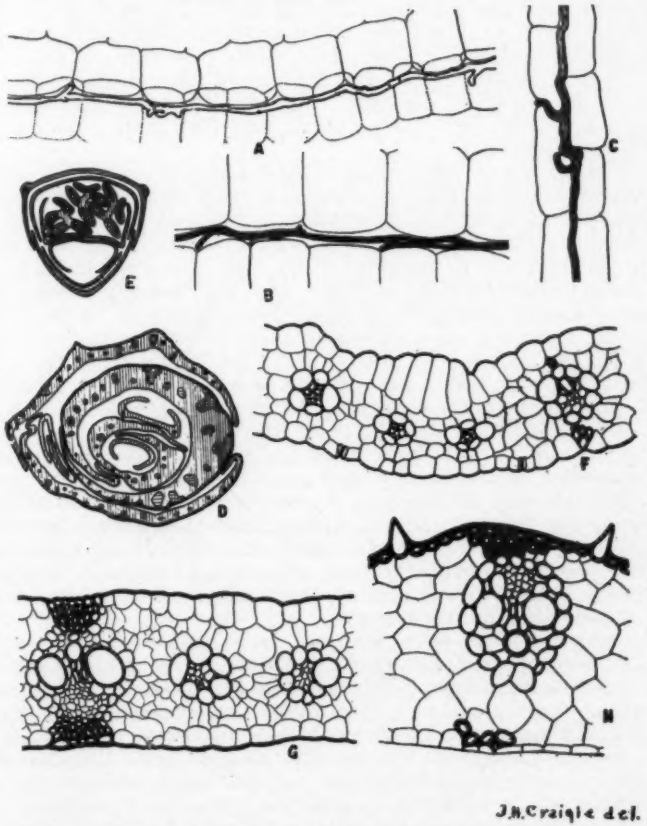

FIGURE 4.—Details of internal structure of malformed spikelets: A, Thick-walled strand of mycelium without cytoplasmic content, showing swellings and prominences which press against the cell walls. × 200. B, Thick-walled strand of mycelium with content. The host cells have been wedged apart by the mycelium. ×200. C, Portion of thin-walled hyphal strand with cytoplasmic content. ×200. D, Schematic representation of a cross section of a deformed spikelet, cut 3 mm. above the base, to show the inrolled nature of the lemmas. In this spikelet an additional abnormality appears in the form of an extra floral bract just inside the glumes. The vascular bundles and sclerenchymatous tissue are crosshatched. ×about 14. E, Schematic representation of a cross section of a healthy spikelet. × about 14. F, Portion of a cross section of a virescent lemma, showing its similarity in internal structure to the normal leaf. × 200. G, Portion of a cross section of a healthy leaf. × 200. H, Cross section of a portion of strongly inrolled lemma at its thickest part, showing the external thick-walled epidermis, the sclerenchymatous reinforcement cells which connect with the bundle, and the thin-walled inner epidermis with its adjoining small group of thick-walled cells. × 200

occurred sparsely. Abutting on the epidermal cells and just over the bundles were one or more layers of thick-walled cells. Where the bundles were large and more complete, these cells bridged the space between the epidermis and the bundle sheath (fig. 4, H), but

where the bundles were more or less imperfect, thin-walled cells usually intervened between these thick-walled cells and the bundle. On the opposite side of these bundles and lying adjacent to the inner epidermis there were usually a few thick-walled cells, but these never connected with the bundles. (Fig. 4, H.) The walls of the inner epidermis were thin, like those of the mesophyll cells, and few stomata or hairs were evident on this side. This condition—a firm outer epidermal layer, reinforced by clusters of thick-walled cells and opposed on the inside by only a thin-walled epidermis—seems to account satisfactorily for the inrolling that took place in so many of the diseased lemmas.

As already stated, the lemma of the second floret of the spikelet was either partly or wholly enveloped by that of the first floret, and as an apparent consequence (although it duplicated in a general way the features already indicated of the enwrapping lemma) these features were not so pronounced. The thick-walled outer epidermis, the strengthening clusters of fibers, and the vascular bundles were present, but less conspicuously developed. However, in those cases where its upper portion became uncovered and thus exposed to sunlight, it took on the characters of the enwrapping lemma, which seemed to indicate that if this lemma had had an exposure to sun and air it would have been identical with the one that inclosed it. The leaflike appearance of two or three of these has been mentioned.

The palea of the first floret participated somewhat in the general hyperplastic condition and became frequently three times its normal size, but in no case did it approach the extravagant dimensions of the corresponding lemma. In the second floret, the palea, surrounded as it was by two lemmas, was apparently stifled and only rarely attained the size of the other palea. As a rule, it was poorly developed, often only a vestige being present, and it was more frequently absent than any of the other floral bracts.

The stamens, more often than not, were suppressed. If present, the anthers appeared atrophied and were rarely more than two-thirds their normal size. The pollen grains varied greatly in size and shape, some being even larger than the normal healthy grains, while others, again, were reduced to half that size. (Fig. 3, I, J.) All of the pollen grains in a diseased plant were practically devoid of content, so that the walls became wrinkled and infolded, and the appearance of the grain was irregular and bizarre. (Fig. 3, I.) None of the modifications that Butler (*5*) described for the stamens of *Pennisetum typhoideum* attacked by *Sclerospora graminicola* (Sacc.) Schroet. was observed.

As the characteristics of the mycelium of this fungus have been described by the senior writer in a previous publication (*54*), it is unnecessary to repeat them here. It might be added, however, that the hyphal filaments that seem to serve for communication between different parts of the host were frequently, though not always, very thick walled, a condition resembling that observed by Butler (*5*) in *Sclerospora graminicola* on Pennisetum. Usually these filaments were devoid of cytoplasmic content. A portion of a thick-walled hyphal filament without content (A) and a portion of one with content (B) are shown in Figure 4, as well as a thin-walled portion of a filament (C).

DISCUSSION

These cases of malformation involve several points of interest. Not only are they remarkable because of their rarity in the Gramineae in general and in teosinte in particular, but also because of their elaborate structural overdevelopment in contrast to their complete sterility, their growth as independent plantlets, and their resulting abortive apogamy. Moreover, they present an interesting comparison to cases of malformations in other grasses and contribute to possible interpretations of such structures and their significance.

In the first place, these malformations are of interest because in teosinte the occurrence of structural abnormalities of any kind, whether resulting from injury by downy mildew or from any other factor, is exceedingly unusual. Although numerous plantings of teosinte from several lots of seed obtained through the kindness of G. N. Collins and others from Mexico, Florida, and other localities were kept under observation for two years in the Philippines, the senior writer found exceedingly few and slight abnormalities either in healthy or in downy-mildewed plants with the exception of the present cases. Moreover, Collins, who has studied extensive plantings for many years, summed up his long experience in a lecture in 1918 (*12*) with the statement:

> Compared with maize, teosinte is absolutely constant. This is true whether looked at from the standpoint of fluctuating variations of a quantitative nature or the frequency of occurrence of abnormalities. We have had teosinte under observation every season for 10 years, and during that time, with the exception of some apogamous plants one season at San Diego, not a single pronounced abnormality has appeared.

Nine plants survived the crowded conditions in the senior writer's test plot in the Philippines. That the six infected ones of these all developed decidedly malformed tassels is therefore the more striking. It should be noted in this connection that from seed of representative plants in the parent plot Collins made certain that this teosinte was pure and free from contaminating hybridization with maize.

In the second place, these malformations are of interest in themselves because they show such remarkable modifications in structure. The elaborate overdevelopment of such floral parts as the glumes and lemmas; their hypertrophy to many times the normal extent and bulk; the formation of additional fibrovascular bundles within them; the marked alteration in size, position, arrangement, and relation of the florets and their parts; the local unevenness of growth with consequent crumpling and curling of mature organs; and the binding and restraint of rapidly expanding inner portions of the inflorescence by the adherent unexpanding outer parts enfolding them, with consequent contortion, buckling, and tearing—all these features mark these malformed inflorescences as noteworthy cases of abnormal growth. As such they may be of interest in the field of teratology, a branch of botany little emphasized at present, but one in which in the past Penzig (*44*), Masters (*33*), Moquin-Tandon (*37*), and others not only devised extensive classifications and terminologies, but also made comparative studies of the structural modifications in different groups of plants and worked out convergences and homologies that are still of some general significance. The extensive deformation of these teosinte tassels is of added interest because it seemingly was consequent to the irritation of the downy-mildew mycelium growing immediately within their tissues, an action which apparently

in most cases was direct, as in very few instances did the apparent absence of hyphae within the deformed floral parts permit consideration of possible action at a distance.

In the third place, these malformed inflorescences are of interest because they were completely sterile. This was true not only in cases where the spikelets entirely lacked sexual organs and were profoundly modified, but also in cases where the florets in general appearance were almost normal and developed stamens, for the pollen was invariably abortive and nonfunctional. Moreover, they were sterile with respect to both sexes, for in no case did female branches develop. This sterility, however, was accompanied by unusual vegetative rejuvenescence. Uncommonly numerous shoots were developed continuously from the bases of the plants, and when during the heavy storms the plants were beaten down into the mud, they sent up many new sprouts from their nodes. The continued proliferation was so pronounced that during the six months they were under observation the plants, although of the annual species of teosinte, in general resembled the perennial type that occurs in Mexico and has been studied and described by Collins (*14*). That these plants should be sterile florally and yet have such unusually prolific vegetative growth gave rise to the rather anomalous situation that if the plants had been grown for seed or to furnish pollen for hybridization the effect of the downy mildew would have been regarded justly as very injurious, quite as it is in the case of other grain-bearing crops such as maize or wheat; whereas, if the plants had been wanted for their vegetative growth for fodder or stover, the effect of the mildew on the whole was beneficial, rendering them even more valuable than the less prolific healthy individuals.

It is of interest also that coincident with the sterility of these plants there took place an abortive apogamous reproduction through the temporarily independent growth of the seedlinglike spikelets, for although examples of this viviparous method of propagation have been observed in the Gramineae they apparently are rare in the tribe Maydeae. The cases of apogamy in maize reported by Collins (*9*) are the only ones in the tribe described hitherto, and the cases in teosinte (unfortunately not described but merely mentioned in the same paper) are the only ones ever even recorded in that genus, as far as the writers have been able to ascertain.

In the fourth place, malformations such as these in teosinte may perhaps have some structural or phylogenetic significance as traumatic reversions to ancestral conditions. The consistently leaflike structure of the abnormal growths which develop from diverse injurious agents in the several Gramineae considered probably may be interpreted as evidence for the theory that the leaf is the ultimate progenitor from which all floral organs have been evolved.

Is it possible to go still farther? In a comparable case—an intensive study of the malformations of maize following attack by corn smut (*Ustilago maydis*)—Iltis (*22*) has found structural evidence which he considers to indicate beyond doubt that Zea has been derived indirectly from the Andropogoneae. This might arouse expectations that these malformations of teosinte may offer some structural evidence of significance in the phylogenetic question of the ancestry of teosinte and even of the ancestry of maize, with which teosinte is more or less involved in the hypotheses advanced by Collins

(*10, 12, 15*), Montgomery (*36*), Weatherwax (*53*), and others. Yet toward a solution of the disputed question whether maize originated by slow evolution or as a mutation or sport from teosinte or some ancestral plant like it; or whether it arose as the result of hybridization between teosinte and some other grass; or whether pod corn, erroneously believed to grow wild in Paraguay, is the ancestor of cultivated maize quite separate from teosinte, these abnormal inflorescenses of teosinte seem to furnish no clear evidence.

To be sure, these malformed teosinte spikelets somewhat resemble those of pod corn (*Zea tunicata*) in the overgrowth of their glumes, a character well developed in this type of Zea, not only in the ear but also at times in the tassel. (Collins *11, pl. 13, B*). This resemblance, even though some consider pod corn as the type most primitive or most like ancestral maize, can hardly be of any special significance, however, for similar overgrowths of floral parts occur in such genera as Pennisetum and in other tribes too remote to furnish any evidence of ancestral characters or relationships in the Maydeae. Likewise, there seems to be no significance in the resemblance between the adherence and binding of the leafy parts in certain deformed teosinte spikelets (fig. 2, C, D) and that traced by Kempton (*27, fig. 16*) as an inherited feature in "adherent" plants of certain strains of maize, and between the lobing and consequent entanglement of parts in the deformed teosinte tassels and that which Kempton (*26*) has found in leaves, husks, and tassel glumes, not only in maize but also in teosinte and related grasses.

Moreover, Collins (*14*) has suggested that the perennial type of teosinte is the more primitive from which the annual has been derived (possibly by crossing with its annual relative, maize). The unusually abundant and prolonged production of new shoots by the infected teosinte plants might be interpreted as a reversion to an ancestral condition. More probably, however, it is merely an example of the prolongation of vegetative growth which often follows tolerated parasitism of Sclerospora in other hosts (*57, pl. 5*).[4]

In the fifth place, these malformations in teosinte present interesting comparisons to those recorded in cases of infection by the other species of Sclerospora in numerous grasses. In the case of the common *Sclerospora graminicola*, for example, malformations of the inflorescence of an extensive list of grasses, chiefly in the tribe Paniceae, have been reported many times from Europe, Africa, China, Japan, and India. Also, the widely distributed *S. macrospora* has been found inducing deformities of the floral structures in some 25 hosts (including maize, wheat, rice, and many wild grasses) in 9 of the 11 gramineous tribes in many localities through Europe, Asia, Australia, and the United States. In the other species of Sclerospora, moreover, although less numerous, striking, and widespread, similar cases have been reported, so that in all more than 40 papers might be referred to as pertinent. From these facts it seems obvious that these plants of teosinte, in showing malformations of the inflorescence as the result of attack by downy mildews of the genus Sclerospora, were only behaving toward these parasites as many other genera of grasses have

[4] Further resemblances of possible phylogenetic significance might be noted, but realizing that such material can be better interpreted by others more familiar with the morphology, development, genetics, history, relationship, and agronomy of maize, teosinte, and their relatives, the writers will gladly turn over the remaining material, including slides, photographs, and notes, to those who will undertake further study.

been found to behave in the history of our knowledge of this group. On going over these cases and comparing the deformities in the tassels of teosinte with those developed in other male inflorescences, such as the tassels of maize (*17*, *19*, *23*, *25*), and those formed in the perfect spikes or inflorescences of other grasses, such as Pennisetum (*5*, *8*, *29*), Setaria (*20*, *21*, *29*, *34*, *48*, *49*, *51*, *58*), Triticum (*1*, *2*, *4*, *39*, *40*, *41*, *43*, *47*, *52*, *55*), Oryza (*18*, *59*), Agropyron, Phragmites, etc. (*42*, *43*), it is noticeable that there is a general similarity, but that these in teosinte are on the whole more extensive in their growth, more elaborate and complex, and more profoundly altered in their structure. In their behavior, that is, in the partially successful growth of some of the more pronouncedly modified spikelets as if they were young independent plants, they are in some ways unique among the cases of abnormalities resulting from Sclerospora attack. It seems probable, however, that some of the elaborately malformed spikelets in the virescent tassels of maize infected with *S. macrospora* described in Italy by D'Ippolito and Traverso (*25*) and others would have made at least abortive attempts at independent apogamous growth if they had been given opportunity.

Finally, when the foregoing points have been considered, what seems to be the most probable interpretation of such malformations as these in teosinte? All deformed inflorescences in the case of wild and cultivated Gramineae are not occasioned, of course, solely by the growth of Sclerospora in the host tissue. Deformities with general resemblance to those just considered have been found, for example, to develop as the result of attack by nematodes in wheat (Leukel, *30*) and rice (Butler, *7*); of infestation by insects in wheat and Setaria (D'Ippolito, *24*); of chemical and mechanical injury in maize (Blaringhem, *3*); and even, apparently, of excessive moisture in wheat (Lo Priore, *31*); probably following the general principle emphasized by Knox (*28*) that most malformations such as phyllodies, multiplications, fasciations, torsions, or virescences arise from injuries and are not heritable. Also, in a few instances malformations of the inflorescences very similar to these of traumatic origin have been found by Kempton (*27*), Zapparoli (*60*), and others to develop in maize unassociated with any detectable injury and to persist as hereditary characters.

These points seem to suggest the following: Under normal conditions the floral parts of teosinte and other grasses have inherent possibilities of certain structural development regarded as usual, normal, or typical. Also, however, they have inherent potentialities for ways of development that are unusual, nontypical, or abnormal, but may be induced by the action of various factors that are injurious or that disturb the usual course of growth. Obviously, there are limits to the inherent capabilities for abnormal development; but within these limits they show great range in such structural excesses as remarkable leaflike elaboration, extensive overgrowth of floral envelopes, and suppression of sexual organs; and these abnormalities, agreeing in general, may be called forth in response to various diverse disturbing influences. Under ordinary conditions of growth in the field, however, such disturbing influences are encountered but seldom; consequently, such malformations develop only rarely. Under ordinary circumstances, also, cases of malformation attributable to

chemical injury, to harmful environmental conditions, or to noxious insects are relatively infrequent.

Attack by Sclerospora, however, is a disturbing factor encountered in such a wide range of valuable and much-studied host crops, in so many different parts of the world, and it is so commonly followed by malformation of the inflorescence, that such deformation very naturally has come to be regarded as one of the distinctive symptomatic stigmata of these diseases.

SUMMARY

This paper considers the case histories, structural peculiarities, and possible interest or significance of malformed male inflorescences of teosinte (*Euchlaena luxurians*) infected with the downy mildew (*Sclerospora philippinensis*) in the Philippines.

The tassels were strikingly altered in appearance and structure; the spikes shorter, the rachis in extreme cases comprising only a few joints; the floral elements progressively reduced in size and number from base to tip of spikes, the tip spikelet in some represented by a single glume; the glumes, and particularly the lemmas, in reduced spikelets, excessively hypertrophied, the latter usually characteristically enwrapping although occasionally expanded above to a green blade, leaflike in gross structure and in histology; the paleas less hypertrophied, sometimes in the second florets only vestigial; the stamens generally wanting, but when present having atrophied anthers and abortive pollen. Not only were the tassels profoundly malformed, but the female inflorescences were suppressed, so that the plants were completely sterile. This sterility was accompanied by unusually prolific vegetative growth, the continued and abundant production of new shoots showing some resemblance to the growth habit of perennial teosinte.

In tassels left lying on the ground during heavy rains, deformed spikelets comprising leafy shoots resembling seedling plantlets remained green and vigorous, grew into an upright position, sent out roots from their bases, and when transplanted continued to develop independently for more than a week. Within their tissues the mycelium of the fungus remained living and apparently capable of continued growth had the plantlets lived. As such cases of apogamy or vivipary are unusual in the gramineous tribe Maydeae and exceedingly rare in teosinte, these are described and illustrated, and compared with those following infection by other species of Sclerospora in other grasses.

The possible bearing of these malformations on the general question of the interpretation of floral malformations is outlined and their possible significance is considered.

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PROPAGATION AND FOOD TRANSLOCATION IN THE COMMON MILKWEED [1]

By FISK GERHARDT [2]

Assistant Chemist, Iowa Agricultural Experiment Station

INTRODUCTION

The recent interest in the milkweed (*Asclepias syriaca* L.) has centered around its possible commercial use. It has been investigated as a possible source of rubber from its latex, oil from its seeds, fiber from its vascular elements, and coma from its fruit. Little attention has been given, however, to the movement of food materials within the plant, and where it has been studied the major interest has been confined to changes which occur within the individual organs, such as the root, leaf, stem, or fruit.

The present investigation was undertaken to gain an understanding of certain physiological processes which affect successful propagation, together with the formation of various organic constituents and their translocation at frequent intervals throughout the vegetative and dormant period.

REVIEW OF LITERATURE

In the milkweed, vegetative propagation is accomplished by adventitious buds appearing along the surface of a deeply penetrating root system (*26*).[3] In the edible canna, Ripperton and Goff (*31*) contended that vegetative viability depends upon the condition of the adventitious bud rather than upon the root stalk proper.

According to Crocker (*6*), weed seeds have a high vitality, especially the more noxious weeds and those in which the seed coat secures a long delay. However, this is contrary to the statement of Duvel (*10*) and others, who failed to emphasize the seed-coat characters in their study of the vitality of weed-seed embryos. Imperviousness of seed-coat characters to oxygen and to moisture has been stressed in the work of Crocker (*6*, *7*), Shull (*36*), and Harrington (*15*, *16*, *17*), while Pack (*25*) has shown that proteins decrease in the seed coats during afterripening. Merlis (*21*), Schmidt (*33*), and Kirkwood (*20*) have indicated a transformation of oil to sugar in fatty seeds. Certain seeds, such as the guayule (*20*) and hawthorne (*6*), however, fail to germinate under any circumstances until after a long period of dormancy. In such instances the embryo is regarded as immature, and certain changes prior to germination are required.

In spite of the wide natural distribution of the common milkweed, no record has been found relative to the germination of its seed. In the present paper attention is confined to the germinational response of milkweed seed under various environmental conditions.

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[3] Reference is made by number (italic) to "Literature cited," p. 849.

Studies upon the elaboration and movement of food materials in plants have been approached from several different points of view. In the leaf tissue these phenomena have received the attention of Brown and Morris (*4*), who found sucrose the primary product of synthesis and hexoses the primary product of translocation. Sucrose predominated in young mangold leaves, while later hexoses increased throughout the entire leaf with an accumulation at maturity (*8*). The work of Parkin (*29*) on the snowdrop and of Colin (*5*) on the sugar beet substantiates the claim that sucrose is the first sugar formed. This sugar is then reconverted into hexoses for translocation to the root, and there in the presence of an enzyme it is converted to starch, inulin, or sucrose, as the case may be. Although starch is absent from the leaves of several plants (*8*, *9*, *22*), Schimper (*32*) concluded that hexose formation preceded that of starch and that the latter is only formed when glucose reaches a fixed maximum limit, which differs for each species.

Attention also has been given to the change in quantity and composition of food material translocated during different stages of maturity. Shaw and Wright (*35*) found total proteins decreased in both sunflower and corn throughout the growth period. While starch remained constant in the case of the sunflower, it increased twentyfold in the corn plant. The largest amount of carbohydrate and ash material was found to be present in the timothy plant during its early stages of development (*13*), while nitrogen and ether extract increased during maturity. Nitrogenous material was stored chiefly in the roots, but no starch was present during the storage process. In the wheat plant (*13*, *19*, *39*), nitrogen and carbohydrates appear to be the materials which the leaf and stem yield to the maturing head. During late fall as the sweetclover enters dormancy, nitrogen of the tops decreases and is stored in the roots (*37*). During active growth the largest amount of nitrogen was found in the tops, the maximum appearing during the month of June.

Although the milkweed possesses certain other commercial possibilities (*24*), interest in this plant has largely centered around its value as a source of rubber (*11*). Fox (*12*) indicated that the rubber content varies somewhat with the amount of available soil moisture, while Hall and Long (*14*) found that ecological factors affect rubber formation. No analyses, however, have been reported concerning changes in rubber content with progressive maturity of the plant.

In the present investigation it was deemed advisable to follow the movement of organic and inorganic materials in the milkweed by making separate analyses of the roots, stems, and leaves at stated intervals over a period of one year. Movement of food reserves in the different organs could thus be correlated with each other as well as with progressive maturity of the entire plant.

EXPERIMENTAL STUDIES

PROPAGATION BY SEED

Preliminary observations were conducted upon native plants, but it soon became apparent that it would be necessary to propagate the milkweed in order to obtain a sufficient supply of experimental material. For this work a plot, comprising approximately 100 square feet, situated 2 miles south of Ames, Iowa, was selected.

During the fall of 1925 a pint of seeds was collected from native plants. These were dried in paper bags in the laboratory until the following spring, at which time they were scarified. There occurred an average germination of 85 per cent. During the week of May 1, 1926, a pint of scarified seed was used to plant about one-twentieth of an acre in rows 3 feet apart. Because of excessive drought germination was slow, but by June 15 the young plants had attained a height of 5 inches and required thinning. During the first year five plants of the entire lot blossomed and produced seed, thus showing unmistakably that they were annuals. These were noted and the seed from them was collected separately the following year. A

FIGURE 1.—Milkweed plants dug at Ames, Iowa, May 4, 1927. Vegetative propagation occurs from the previous year's roots

killing frost during the first week of October, 1926, resulted in the shedding of all leaves and the cessation of further growth and development.

In the spring of 1927 active growth of the plants in the field plot, as manifested by their appearance aboveground, began early in May. The condition and mode of formation of new plants may be seen in Figure 1. A large number of adventitious buds appear on the rootstocks of the previous year. In the breaking up of these older rootstocks, the distribution of the milkweed may actually be increased instead of curtailed. Figure 2 shows the field plot, on July 14 during the late-blossom stage. Sterility resulted from attempts to self-pollinate the five annual strains previously mentioned. Cross-fertilization by such agencies as wind and insects evidently is the rule.

Seed Germination

Detailed studies were made on the germination of seed under different conditions. Prior to a killing frost, seed was collected from native milkweed grown during the summer of 1925. When placed in germination chambers the fresh seed showed little response. Rapid germination was not attained until after the seed had been stored for approximately four months. This fact indicates that milkweed seed must pass through a rather protracted period of afterripening.

To determine the practice necessary to obtain maximum germination, a study was made of the germination of the seed as influenced by chemical treatment, temperature, and storage. In some instances the seed used had been stored in the laboratory for 12 months, in others for only 2 months. Its response to temperature, seed-coat mutilation, and oxygen is summarized in Table 1.

Table 1.—*The effect of treatment and storage upon germination of milkweed seed*

Specific treatment	Temperature during time of germination	Period of storage	Germination
	° C.	*Months*	*Per cent*
None	20–25	12	21
Do	20–25	2	14
Kept at 10° C. for 5 days	20–25	12	32
Kept at 10° C. for 8 days	20–25	2	14
Exposed to concentrated H_2SO_4 for 3 minutes	20–25	12	40
		2	23
Exposed to concentrated H_2SO_4 for 6 minutes	20–25	12	54
		2	20
Scarified	20–25	12	75
		2	24
Scarified, and kept at 10° C. for 5 days	20–25	12	92
		2	40
Kept in an atmosphere of oxygen	20–25	12	46
		2	12
Kept in an atmosphere of oxygen 10° C. for 5 days	20–25	12	59
		2	42
Oxygen scarified	20–25	12	85
		2	24
Oxygen scarified, and kept at 10° C. for 5 days	20–25	12	91
		2	70

A study of this table shows that milkweed seed under natural conditions possesses low germinating power even though the vitality of the embryo may remain high. Seeds that are completely afterripened give a decided response to scarification; but only a slightly higher germination was attained in an atmosphere of oxygen. It may be concluded, then, that mutilation of the testa increases the imbibitional capacity of the embryo for moisture rather than for oxygen. Alternating temperatures favor germination even in afterripened seed. This is true also after scarification, and indicates that in milkweed the change caused by alternating temperatures is not confined to the seed coat alone but is shared also by the embryo.

No decided response to scarification was noted in seed stored for two months and germinated at room temperatures. Evidently the seed-coat characters alone are not responsible for the long period of afterripening. In this case 14 per cent of the untreated seed germinated at room temperature and no increase was obtained from

scarification. However, when the seed was subjected to increased oxygen supply and alternating temperatures together with scarification, the germinational response rose to 70 per cent. In general low temperatures inhibit respiration, producing a low respiratory ratio which permits a storage of oxygen in the seed and tends to hasten afterripening. Afterripening is also hastened by increased oxygen pressure, alternating temperatures, and scarification.

VEGETATIVE PROPAGATION

In the fall of 1925 a number of plants were lifted from the soil with as much of the root adhering as was possible and taken to the greenhouse, where a medium temperature was maintained. The first plants were transferred on October 3. Additional plants were dug

FIGURE 2.—Experimental milkweed plot, blossom stage, Ames, Iowa, July 14, 1927

on November 5, December 4, and on January 18 of the following year. The roots were placed in 7-gallon earthenware jars. One-half of the plants were set in a sand medium and the remainder in clay loam. In the October root collections a period of six weeks elapsed before growth began, while in the subsequent propagations the time interval became proportionately shorter, until in the January plantings only two weeks was required. These results suggest that the roots undergo a well-defined rest period. No attempt was made to shorten the rest period by the use of chemical reagents. In all cases the plants in sand cultures responded more rapidly to treatment than those in the clay-loam medium. In both series the amount of stored food material in the roots was the same. The greater growth response in the sand cultures was due possibly to better aeration. Normal growth, however, was not attained in the greenhouse, and when the plants were transplanted to an out-of-door plot in the spring they

were no further advanced after a few weeks than those which came up under normal environmental conditions. The greenhouse procedure, therefore, offered little encouragement as far as increased development was concerned. Since the roots of the milkweed undergo a definite period of rest, vegetative propagation is possible, without an appreciable loss of time, from roots which have been allowed to remain out of doors all winter.

FOOD TRANSLOCATION IN THE PLANT

A study was made of synthesis and translocation and storage of food materials to ascertain the changes in these processes that take place in the plant at different stages of maturity. Chemical analyses of plants were made at definite intervals. The plants used had been grown the previous year from native seed. Samples of the roots were collected once a month from November, 1926, to November, 1927, inclusive. Samples of the green portion of the plant were collected at stated intervals from May, 1927, to November, 1927, inclusive. The plants succumbed to a killing frost in the early part of October. The samples taken in November were therefore indicative of post-mortem composition.

Chemical Analyses

Immediately after collection the samples were brought to the laboratory, cut into 1-inch pieces, placed in a Freas electric vacuum oven, and desiccated at 65° C. in a current of air. The air-dried material was ground until it could be passed through a 100-mesh sieve. Chemical analyses were made upon this material by the methods described below.

Moisture determination.—A tared sample of ground tissue was dried to constant weight in a Freas electric vacuum oven at 70° C.

Fat.—The residue from the moisture determination was freed from lipoids and soluble pigments by percolation with anhydrous alcohol-free ether. The ether extract was dried to a constant weight in an electric oven at 100° C. and expressed as percentage fat.

Reducing sugars.—After expulsion of the ether, the residue of the sample was extracted with boiling 90 per cent alcohol (to which a small amount of $CaCO_3$ was added) for one-half hour on a hot plate. The filtered extract was concentrated to remove alcohol, diluted with water, defecated with neutral lead acetate, freed from excess lead by sodium carbonate, and finally made up to definite volume. Reducing sugars were determined on aliquot portions by the Quisumbing and Thomas (*30*) method and the results expressed as percentage dextrose.

Total reducing sugar.—Aliquot portions from the original sugar extract were hydrolyzed with boiling 2.5 per cent HCl for one hour. Total reducing sugar determinations were carried out and the results expressed as in the case of simple reducing sugars.

Starch and dextrin.—The residue from the sugar extraction was boiled with 150 c. c. of water for two minutes in order to gelatinize the starch. After being cooled to 38° C. and digested with fresh saliva until a negative result was obtained with iodine, the filtered solution was hydrolyzed with 2.5 per cent HCl for 2.5 hours. Glucose was determined as above. The results were multiplied by the factor giving the equivalent values for starch and dextrin and expressed as percentage of starch. This figure actually includes the amount of water-soluble pentosans and any other soluble or partially hydrolyzed products possessing reducing power.

Hemicellulose or acid-hydrolyzable material.—The residue from starch digestion was boiled with 150 c. c. of 2.5 per cent H_2SO_4 (by weight) for one hour. The filtered solution was neutralized and clarified in the usual manner. The

reducing power of this group of carbohydrates was determined (*30*) and expressed in percentage of glucose.

ASH.—A 2-gm. sample was ashed at a dull red heat in an electric furnace to a constant weight.

PENTOSE.—Two-gm. samples were distilled with 12 per cent HCl according to the Official Methods (*1*). The furfural phloroglucid value was multiplied by the proper equivalent and expressed as percentage pentose.

TOTAL NITROGEN AS PROTEIN.—The total nitrogen present in a 2-gm. sample was determined by the Kjeldahl method and the result multiplied by the factor 6.25 to represent total nitrogen expressed as crude protein.

ACETONE EXTRACT.—A 2-gm. sample was extracted 24 hours on an electric hot plate with chemically pure acetone. The weight of the evaporated extract, containing gums, resins, and pigmented material, was expressed as percentage acetone extract.

BENZENE EXTRACT.—The residue from the acetone removal was extracted 24 hours on an electric hot plate with chemically pure benzene. The soluble product (rubber) was expressed as percentage benzene extract.

In the presentation of the chemical data all percentages are calculated upon air-dry basis.

The data pertaining to the chemical composition of the leaves and stems are presented in Table 2 and in Figures 3 and 4. The total nitrogen content of both leaf and stem tissue of the young, succulent plant remains high, that of the leaf surpassing that of the stem during May and June. With an increase in maturity the leaf loses more than 70 per cent and the stem 82 per cent of its nitrogenous constituents. Although the ash content of the stem decreases during the summer months, inorganic constituents again accumulate during early fall. The leaf shows a progressive increase with maturity, the ash content being more than twice as great in November as in May. The amount of gum and resins, as indicated by the acetone extract, represents from 15 to 25 per cent of the total dry weight of the tissues. These substances are found in larger amounts in the green portion of the plant, predominately in the stem.

TABLE 2.—*Percentage composition of milkweed leaves and stems during growth cycle*

LEAVES

Date of sampling	Moisture	Total nitrogen as protein	Ash	Acetone extract	Benzene extract	Reducing sugar	Total sugars	Starch * and dextrin	Acid hydrolyzable material	Fat	Pentoses
May 15	2.58	32.55	8.90	14.45	0.49	4.83	15.10	0.95	5.48	5.75	9.52
June 5	2.20	29.39	9.50	13.75	.60	5.84	13.02	1.75	6.52	6.30	10.53
June 23	2.75	17.50	10.90	15.00	.81	5.28	11.28	.95	7.40	6.40	12.13
Aug. 5	3.50	13.45	14.10	13.45	1.50	2.17	8.48	.00	6.50	8.18	10.55
Sept. 7	2.30	12.77	16.60	14.68	2.55	1.95	8.50	1.08	5.48	10.45	10.02
Oct. 6	2.50	10.93	17.30	12.98	2.85	.68	3.70	1.07	7.76	11.35	12.03
Nov. 10	2.35	15.75	19.10	11.20	.82	1.80	3.74	2.49	8.28	8.00	10.73

STEMS

May 15	2.26	23.50	8.45	22.70	0.30	15.68	20.56	1.27	7.49	4.40	10.02
June 5	3.72	15.05	7.00	25.60	.27	15.08	21.88	.92	7.60	3.25	13.20
June 23	3.18	9.72	5.15	20.25	.47	9.68	16.48	1.40	10.00	3.05	15.82
Aug. 5	3.45	6.56	2.80	18.30	.40	6.88	10.20	.32	11.48	2.40	19.06
Sept. 7	2.85	5.05	3.10	17.14	.30	8.25	14.08	.75	11.04	2.50	20.33
Oct. 6	3.00	3.97	4.50	12.20	.31	5.02	7.13	1.30	14.72	2.15	21.25
Nov. 10	3.65	4.20	5.70	7.50	.30	.88	1.00	.00	15.76	2.75	21.64

* No iodine reaction for starch, the small amount of reducing substances here represent dextrins and H_2O soluble pentosans.

Rubber (benzene extract) is present in the stems only in traces. Since none is present in the roots, the leaf must remain the center of rubber synthesis and storage. This conclusion is further strengthened by the fact that there is a gradual increase in benzene extract with maturity. Attention should be called to the post-mortem examination as represented by the samples obtained November 10 in which

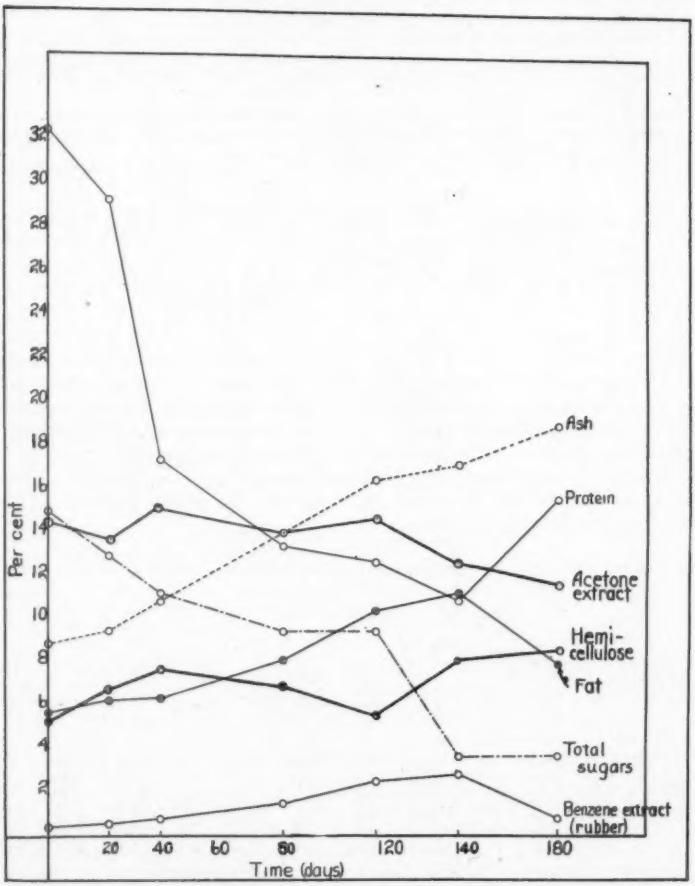

FIGURE 3.—Changes in chemical composition of milkweed leaves during the growth cycle

over four-fifths of the total rubber present had been destroyed by a killing frost. The analyses showed 2.53 per cent rubber in leaves prior to killing frost, 1.54 per cent present five days after the frost, and 0.50 per cent present two weeks after the frost. It is difficult to explain this loss, since no increase was found in the acetone or benzene extract of the stem tissue.

Like nitrogen, soluble carbohydrates appear to reach their maximum concentration in the young tissue. A progressive loss of these soluble constituents was noted. According to Parkin (*27*), starch as a rule is formed plentifully in most dicotyledonous leaves. In the milkweed, however, this was not found to be the case. Evidently the milkweed stores its leaf carbohydrates in the form of sucrose, since this sugar predominates in the leaves. In the stem the predominating sugar is hexose. The total sugar content of the stem is also considerably higher than that of the leaf. Hemicelluloses evidently do not serve as a reserve or storage material in the milkweed leaf, but in the stem they increase with the age of the plant. Approximately 10 to 12 per cent of pentosan material is found in the leaf. The absence of starch and the fairly uniform hemicellulose and pentosan content in the leaf are strong indications that synthesis,

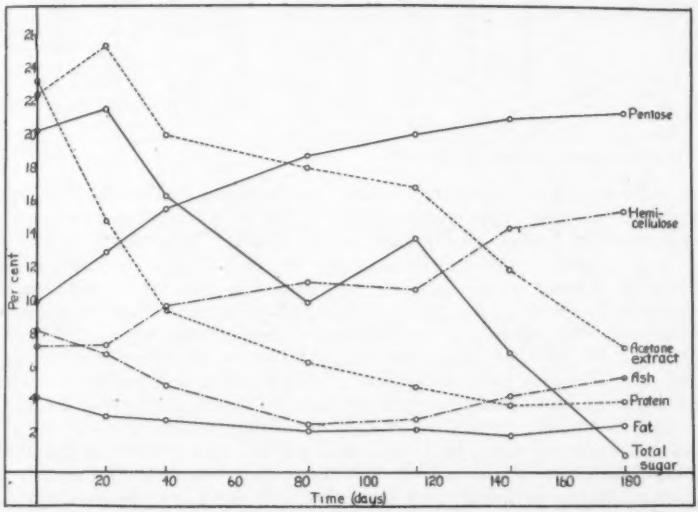

FIGURE 4.—Changes in chemical composition of milkweed stems during the growth cycle

storage, and translocation of carbohydrate material are accomplished through the medium of sucrose.

The results of chemical analyses of the root tissue made at monthly intervals during the year are shown in Table 3 and Figure 5. It is evident that the root system serves as a storage medium for vegetative propagation. Here, as in the leaf and stem, nitrogenous and carbohydrate materials form the chief migratory and storage constituents. The gums, resins, and lipoids are not concentrated in the underground organs and exert probably only a minor influence. Nitrogen migrates out of the root tissue during the period of great physiological activity in the green portions of the plant, and accumulates during the late fall and winter months. Although Murneek (*23*) found hemicellulose to function as a storage material (like starch) in the spur tissue of the apple tree, nevertheless it does not offer such a possibility here since

no decided changes occur in its deposition or utilization. The pentosan content of the root constitutes from 12.5 to 19.65 per cent of the dry weight. Except during the summer months, it shows but little fluctuation. This type of material appears as a storage constituent in the stem only.

TABLE 3.—*Percentage composition of milkweed roots during growth cycle* [a]

Date of sampling	Moisture	Ash	Total nitrogen as protein	Acetone extract	Fat	Reducing sugars	Total sugar	Starch and dextrin	Acid hydrolyzable material	Pentoses
Nov. 3	4.10	7.35	9.18	5.90	2.20	0.52	14.08	16.38	9.25	13.84
Dec. 1	3.60	6.30	10.50	6.15	2.10	.50	18.72	8.56	9.00	15.85
Jan. 8	2.80	8.50	11.17	6.40	2.95	1.40	19.06	6.19	8.90	15.65
Feb. 10	4.00	6.78	11.59	6.70	2.68	1.30	21.84	8.71	8.91	14.43
Mar. 12	4.00	6.60	11.17	6.40	2.40	1.45	21.12	8.97	10.12	16.00
Apr. 10	4.00	7.50	12.77	7.00	2.33	2.30	18.72	8.38	9.52	15.95
May 4	3.40	7.00	12.34	6.35	3.22	1.41	13.40	10.00	8.64	16.00
June 5	3.00	8.18	9.88	8.35	3.35	3.20	8.60	6.80	9.00	19.65
July 1	3.30	9.80	5.90	8.60	4.00	3.00	8.59	11.70	9.52	18.70
Aug. 5	2.60	9.85	4.81	8.30	3.75	3.13	10.04	13.40	8.84	18.16
Sept. 7	3.00	7.00	4.72	5.90	2.50	2.12	9.67	30.56	8.70	14.34
Oct. 7	4.30	7.12	7.80	5.55	2.50	.88	10.56	23.44	8.00	12.50
Nov. 7	2.40	8.70	9.21	6.30	2.80	.80	17.28	15.20	8.60	15.15

[a] The benzene extract was nil in all samples.

It is in the root that starch is first encountered. This fact appears significant because this reserve carbohydrate is found in neither the leaf nor stem tissue, where rubber is present. Sucrose here, as in the leaf, remains the predominating sugar. Starch begins to accumulate during early midsummer and reaches its maximum in September. During the winter months it is converted to sucrose and attains its highest concentration during February and March. This fact is substantiated by the statement of Hooker (*18*) and Blackman (*3*) that the inception of dormancy is usually preceded by a carbohydrate and nitrogen increase in which sucrose and starch present a definite interrelationship. During active growth in the green portions of the plant, ash and rubber are stored in the leaf, hemicellulose and pentosans in the stem, and starch in the root.

Hexose sugar is present in the root only at a low concentration, and the amount fluctuates but little with change of season. Sucrose, on the other hand, varies inversely with starch deposition. The starch which accumulates during the summer is converted to sucrose and is used for the production of new tissue the following spring. Figure 5 indicates the cyclic seasonal interrelationship between total sugars, starch, and nitrogen. Figures 3 to 5 show that during May and June the available carbohydrates are largely trans-located out of the root into the green portions of the plant. Nitrogen is also present in smaller amounts. During June and July flower-bud formation and fertilization occur, and in the period of great physiological activity that accompanies these processes carbohydrate and nitrogenous materials migrate out of the root, leaf, and stem tissues into the floral organs, there to be stored later in the developing seed.

COMPOSITION OF LATEX

The lactiferous system of the milkweed plant is composed of individual latex cells, and structurally is identical with that of the

Urticaceae and Apocynaceae. While little is known regarding the function of such a system, Parkin (*28*) has indicated that it serves in the translocation and storage of food reserves. Weiss (*38*) and Schullerus (*34*), on the other hand, attach little physiological sig-

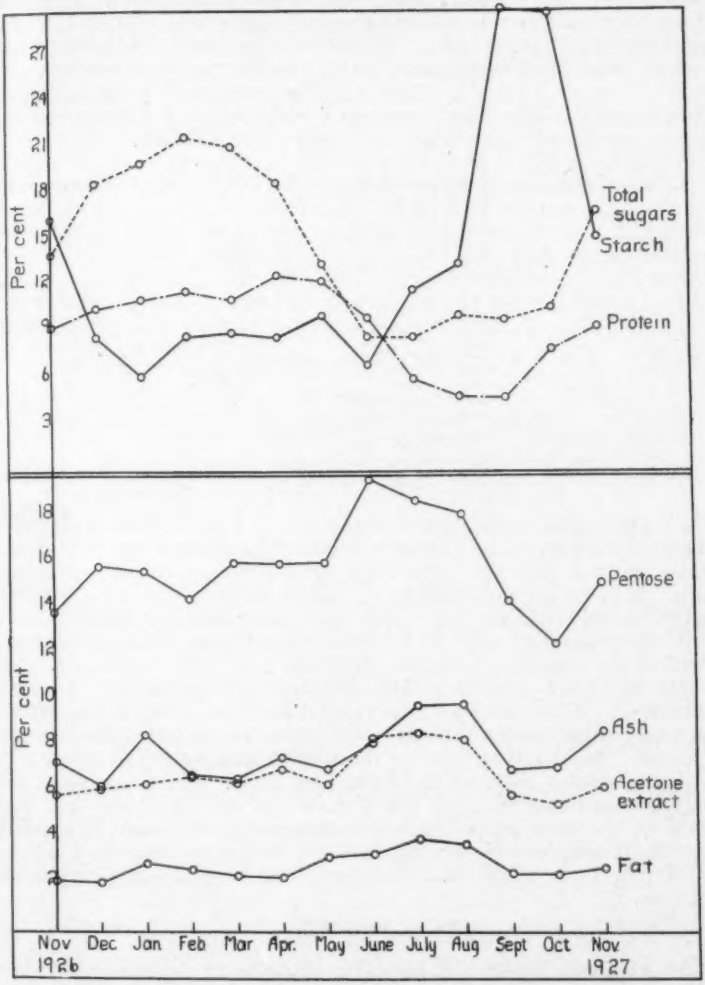

FIGURE 5.—Changes in chemical composition of milkweed roots during the growth cycle

nificance to its presence, believing that it serves merely as a receptacle for the waste products of metabolism.

It was not possible to follow the chemical changes that took place in the composition of milkweed latex during the growing season, since there were not enough plants available to furnish a sufficient

quantity for analysis. However, one collection of latex was made. To obtain the latex for chemical analysis a leaf or portion of the stem was ruptured at several places, and when a drop or two of latex appeared on the surface it was sucked into a small test-tube suction trap, and the rupturing and sucking process was repeated. About 30 plants and some four hours of labor were required for the collection of 20 c. c. of latex. Where the changes in rubber and resin content have been determined, analyses were made of the leaf and stem tissue as a whole. The chemical composition of milkweed latex, collected July 20, as compared with that of a 4-year-old Para rubbertree (*Hevea brasiliensis* L.), is shown in Table 4.

TABLE 4.—*A comparison of the percentage chemical composition of latex from milkweed and from Para rubbertree*

Milkweed	Constituent	Para rubbertree [a]
70.00	Moisture	70.00
1.40	Ash	.26
4.00	Total sugars	.79
23.00	Resins	1.22
3.34	Rubber	27.07
.46	Total nitrogen	.24
0	Disaccharides	
0	Dextrin and starch	
6.52	Hemicellulose	

[a] Beadle, C., and Stevens, H. (*2*).

It is easily seen from Table 4 that milkweed latex offers an inferior source of rubber. The moisture and total nitrogen content of the latex from the milkweed and Para rubbertree approach a common level. It is in the distribution of rubber and resins that a marked compositional difference lies. Milkweed latex contains about twenty times the amount of resin and only one-eighth the amount of rubber found in the exudate of Hevea. The ash and sugar values are from five to six times greater in the milkweed latex than in the Para rubbertree. From the above analyses it is evident that the secretory and compositional mechanism in the two species must be entirely different. Although sucrose is the predominating sugar present in the leaf, hexoses only are found in the latex. Since the latex for analysis was obtained from the midrib and petiole of the leaf and also from the stem proper, in which hexoses predominate, it appears that the laticiferous system might serve as a translocation medium for simple carbohydrates. Nitrogen was present only in small amounts.

SUMMARY

The common milkweed may be propagated vegetatively, but because of a well-defined rest period in the roots this method does not lend itself to practical use. The seed also requires a prolonged period of afterripening. This process is induced by embryological immaturity rather than seed-coat hindrance. Seed-coat permeability is a limiting factor in the germination of fully afterripened seeds. Moisture rather than oxygen is excluded. Alternating temperatures between 10° and 25° C. produced increased germination.

In young, succulent growth nitrogen and carbohydrates predominate. Sucrose is stored in the leaf, translocated in the form of hexoses through the stem to the root, and stored as starch during the late summer, only to be converted back to sucrose at the inception of winter. Leaf, stem, and roots yield nitrogenous and carbohydrate material to the developing flower and seed. Ash and rubber are stored in the leaf, pentosans and hemicelluloses in the stem, and starch and nitrogen in the root. No starch is present in the green portions of the plant.

From the limited trials made, it appears that gums and resins are present in the latex of milkweed in large amounts, rubber only to the extent of 3.5 per cent, and this is localized largely in the leaf. Freezing destroys more than four-fifths of the normal rubber present. A comparison of the latex of milkweed and Hevea indicates a decided compositional difference in the resin and rubber content. Hexoses appear as the only simple sugar present in milkweed latex, which suggests that these lactiferous structures may aid in carbohydrate removal from the leaf to the root, since hexoses are the types of sugar represented in the translocation form.

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EFFECTS OF SOIL TEMPERATURE AND REACTION ON GROWTH OF TOBACCO INFECTED AND UNINFECTED WITH BLACK ROOT ROT[1]

By WILLIAM L. DORAN

Research Professor of Botany, Massachusetts Agricultural Experiment Station

INTRODUCTION

Black root rot of tobacco, caused by the fungus *Thielavia basicola* (Berk. and Br.) Zopf, is known to be much affected by the reaction of the soil. In the field experiments of Anderson, Osmun, and Doran (*3*) [2] this disease did little or no damage in a soil as acid as pH 5.6, and it was severe in a soil with a pH value of 5.9. One year (1925), Anderson and Morgan (*2*) found that this disease was serious only in soils with a pH value of 5.95 or higher, but in the colder summer of the following year, they (*14*) found it in more acid soils and concluded that the safety point, at least for shade tobacco, may be nearer 5.6 than 5.95 on the pH scale. Anderson (*1*) concluded later that no injury occurs below pH 5.6, but that between pH 5.6 and 6.0 there is a doubtful zone within which the root rot may or may not occur.

Black root rot is known to be much affected by the temperature of the soil. Johnson and Hartman (*10*) found that it was most severe at 17° to 23° C., much less severe at and above 26° C., and practically negligible at about 30°. Valleau, Kenney, and Kinney (*21*) reported that at soil temperatures of 21° to 23° C. in the field, black root rot injured tobacco, but that at an average soil temperature of 25.5° C. tobacco grew fairly well in this soil.

In most if not in all previous experiments on black root rot of tobacco, the effects of soil temperature and of soil reaction on the disease have been considered as factors operating independently.

One of the objects of the work described in the present paper was to determine to what extent, if at all, the pH value of the soil at which black root rot of tobacco becomes measurably injurious is affected or changed by the temperature of the soil; in other words, to study the joint effect of these two factors of the environment.

There was in the beginning some reason to expect that the effect of a certain soil reaction on black root rot might itself be influenced by the temperature of the soil; for, according to Jones, Johnson, and Dickson (*12*), the optimum for an environmental factor in infection of a plant by a fungus may vary through some range with other simultaneously varying factors, and Faris (*7*) found that, in the case of infection of barley by covered smut, soil reaction and soil temperature modified the effects of each other. Furthermore, it was suggested by Morgan and Anderson (*14*) and by Anderson (*1*), on the basis of observations in the field, that the exact point on the pH scale at which black root rot of tobacco is a cause of loss may be influenced by the season and especially by the temperature.

[1] Received for publication Apr. 12, 1929; issued December, 1929. Published with the approval of the director of the Massachusetts Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 871.

A clear understanding of the effect of environmental factors on black root rot of tobacco, or on any other plant disease, is possible only if the effect of these environmental factors on the host itself is known. The effects of soil temperature and of soil reaction on the growth of tobacco in the absence of *Thielavia basicola* were therefore investigated also, and the results obtained are here reported.

METHODS

In pot experiments, subsequently described, the constant soil temperatures desired were maintained by means of soil temperature tanks of the type described by Jones (*11*) but modified at the Massachusetts Agricultural Experiment Station. At each temperature there were several pots of soil, each of a different pH value.

For the experiments with *Thielavia basicola*, soil (a stony, sandy loam of glacial origin) was obtained from a field known to be infested with this fungus. Some of the plots in this field had been limed three years before and others had not. Soils of several different pH values were thus available. To secure an even greater range of pH values, the reaction of the soil for some pots was further adjusted with hydrated lime or with sulphuric acid. The amounts of lime or acid necessary to add to produce the desired changes in pH value were determined in preliminary experiments by methods essentially the same as those described by Hopkins (*9*) and Faris (*7*). The pH values were checked monthly,[3] and were found not to change significantly during the course of each experiment.

In three series of experiments soil infested with *Thielavia basicola* was used. In two series of experiments soil sterilized by steam to rid it of this fungus was used in order to study the effect of soil environment on the growth of tobacco in the absence of black root rot.

In all pot experiments, a strain of Havana tobacco susceptible to black root rot was employed. In some cases seeds were sown directly into the pots. If transplants were used, they were taken from sterilized soil to insure freedom from black root rot when set. In one field experiment, strains of root-rot-resistant and root-rot-susceptible Havana tobacco were compared to determine their response to lime in the presence of *Thielavia basicola*.

The results in all experiments are based on the degree of infection of the roots and on the growth, dry weight or leaf areas, of plants.

EFFECT OF TEMPERATURE AND REACTION OF SOIL INFESTED WITH THIELAVIA BASICOLA ON INFECTION OF ROOTS AND GROWTH OF PLANTS

In three series of experiments, unsterilized soil infested with the root-rot fungus was used. Before the results of these experiments are described, however, mention should be made of the effect of soil temperature and reaction on the germination of tobacco seeds and on the period of incubation of black root rot, since the responses in these two respects were essentially the same in each of the several experiments.

SEED GERMINATION

Soil reaction had much less effect on the germination of seeds than on the subsequent growth of the seedlings. Germination was not

[3] Except where otherwise indicated, pH values were determined colorimetrically, by the method used and described by Anderson and Morgan (*2*).

significantly affected by soil reaction within the range of pH values used, pH 4.6 to 6.9, inclusive. This range extends, on the more acid side, below that reported by Van der Poel (*15*, *16*), in whose experiments tobacco seeds germinated well at hydrogen-ion concentrations between pH 5.6 and 7.8.

The relation of soil reaction to germination was not affected by soil temperatures within the ranges used, that is, 15° to 30° C.

Soil temperatures had more effect on the length of time required for germination than on the percentage of tobacco seeds which germinated. The seeds germinated in 4 days at 30° to 21° C., in 6 days at 18° C., and in 10 days at 15° C. Germination, although delayed, was fair at the lowest temperature used, 15° C., which is near the minimum temperature for the germination of tobacco seeds, 13° C., as determined by Johnson and Murwin and reported by Russell et al. (*17*, *p. 16–17*). The best germination was at 30° C., the highest temperature used.

PERIOD OF INCUBATION

Black root rot lesions with *Thielavia basicola* sporulating on them were found in some cases when seedlings were only 2 to 3 weeks old, but no black root rot was found on seedlings less than 2 weeks old.

The colder the soil, down to 18° C., and the more nearly neutral the soil reaction up to pH 6.9, the shorter was the incubation period for black root rot, assuming it to begin in infested soil with the germination of the seeds.

In the more acid soils with pH values of 5.6 or lower, the incubation period was about 10 days longer than in the more nearly neutral soils with pH values of 5.9 or higher. At a given soil reaction, the incubation period was 3 or 4 days longer at soil temperatures of 24° C. or higher than at lower temperatures.

FIRST SERIES

In the first series of experiments black root rot was present only as a trace, or very mild in soils having a pH value of 5.0 or lower at all soil temperatures used, 15° to 30° C. In soils with pH values of 6.5 to 6.9 the disease was severe at soil temperatures of 15° to 24° C., mild or very mild at 27° C., and only a trace was present at 30° C. The degrees of infection of roots were as indicated in Table 1, in which are also recorded the relative sizes of plants, as determined by leaf areas, measured with a planimeter.

At soil temperatures of 15° to 27° C., inclusive, but not at 30° C., plants were much larger in the more acid soils, with pH values of 5.0 or lower, than were plants in the less acid soils, with pH values of 6.5 or above. The greatest differences in sizes of plants, between those in the more acid and those in the less acid soils, were at temperatures of 24° C. and lower.

Black root rot did not retard the growth of plants in soil with a pH value of 5.0 or lower at any temperature. Retardation of growth due to black root rot occurred in soils with a pH value of 6.5 or higher, and this retardation of growth was greatest at soil temperatures of 24° C. or below, less marked at 27° C., and not significant at 30° C. Even in soil as nearly neutral as pH 6.9, black root-rot infection was much lighter and resulting interference with growth much less at 27° C. and at 30° C. than at lower temperatures.

TABLE 1.—*Effect of soil reaction and temperature on black root rot and on growth of tobacco*

[First series]

Soil, pH value	Extent of root rot on tobacco plants of different sizes when grown in infested soil at a temperature of—											
	15° C.		18° C.		21° C.		24° C.		27° C.		30° C.	
	Relative leaf area [1]	Black root rot	Relative leaf area [1]	Black root rot	Relative leaf area [1]	Black root rot	Relative leaf area [1]	Black root rot	Relative leaf area [1]	Black root rot	Relative leaf area [1]	Black root rot
4.6	89	Trace	53	Trace	116	Trace	106	None	108	None	82	None.
4.8	68	do	58	do	104	do	58	do	64	do	62	Do.
5.0	100 / [2] 47	do	100 / [2] 146	Very mild	100 / [2] 224	Very mild	100 / [2] 396	Trace	100 / [2] 517	do	100 / [2] 466	Do.
6.5	18	Severe	12	Severe	9	Severe	10	Severe	36	Very mild	69	Trace.
6.7	19	do	11	do	9	do	12	do	34	Mild	73	Do.
6.9	9	do	6	do	7	do	6	do	33	do	68	Do.

[1] Expressed as relative numbers with leaf areas at pH 5.0 as base.

[2] Leaf area expressed in square centimeters.

TABLE 2.—*Effect of soil reaction and temperature on black root rot and on growth of tobacco*

[Second series]

Soil, pH value	Extent of root rot on tobacco plants of different sizes when grown in infested soils at a temperature of —											
	15° C.		18° C.		21° C.		24° C.		27° C.		30° C.	
	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot
5.0	120	None	73	None	80	None	103	None	106	None	97	None.
5.2	123	Trace	132	Trace	125	do	134	do	100	do	103	Do.
5.6	100 / [2] 0.12	do	100 / [2] 0.86	do	100 / [2] 2.92	Trace	100 / [2] 5.10	Trace	100 / [2] 6.62	do	100 / [2] 9.28	Do.
5.9	88	Severe	22	Severe	21	Severe	22	Severe	33	Moderate	52	Very mild.
6.1	67	do	31	do	18	do	21	do	34	do	56	Mild.
6.3	49	do	26	do	26	do	29	do	32	do	47	Do.
6.5	70	do	27	do	17	do	30	do	37	do	45	Do.

[1] Expressed as relative numbers with dry weights at pH 5.6 as base.

[2] Weight expressed in grams.

In this series, the critical point for black root rot on the pH scale was between pH 5.0 and 6.5 at all soil temperatures at which tobacco will grow below 27° C.

SECOND SERIES

In the second series of experiments, soils with pH values of 5.0, 5.2, 5.6, 5.9, 6.1, 6.3, and 6.5 were used. As before, the soil temperatures were 15°, 18°, 21°, 24°, 27°, and 30° C. The degrees of infection of the roots and the dry weights of plants, the latter expressed in relative numbers, are recorded in Table 2.

Root infection was absent, or present only as a trace, in soils with pH values of 5.6 or lower at all soil temperatures. In soils with pH values of 5.9 to 6.5, infection was severe at soil temperatures of 15° to 24° C. inclusive, moderate at 27° C., and mild or very mild at 30° C.

At all soil temperatures growth was relatively good in soils with a pH value of 5.6 or lower and relatively poor in soils with a pH value of 5.9 or higher. Retardation of growth due to black root rot in these less acid soils was, as compared with growth in the more acid soils, greatest at soil temperatures of 18° to 24° C., less at 27° C., and least at 30° C. A soil temperature of 15° C. is too low for tobacco, and growth was poor at that temperature regardless of black root rot.

In this series, the critical region on the pH scale for infection by *Thielavia basicola* was between 5.6 and 5.9, and this region was not changed by soil temperatures lower than 30° C.

It has often been noted that tobacco plants infected by *Thielavia basicola* in the field tend to grow better or to recover partially as they become older, and this has been attributed to a rise in soil temperature with the advance of the season. While higher soil temperatures are of course important, observations made in this series indicate that some of this recovery may occur without a rise in the soil temperature as the plants become older.

Black root rot in the less acid soils, pH 5.9 to 6.5, retarded the growth of plants more when were young than it did later. Leaf areas were measured with a planimeter when plants in this series were 5 weeks old and again four weeks later. The areas of leaves of plants in the less acid soils, in which black root rot was more severe, were, as compared with the areas of leaves of plants in the more acids soils (pH 5.6 or lower), as follows:

At 15° C., first measurement 33 per cent; second measurement 33 per cent.
At 18° C., first measurement 21 per cent; second measurement 34 per cent.
At 21° C., first measurement 15 per cent; second measurement 43 per cent.
At 24° C., first measurement 9 per cent; second measurement 49 per cent.
At 27° C., first measurement 7 per cent; second measurement 55 per cent.
At 30° C., first measurement 13 per cent; second measurement 67 per cent.

Although the soil temperatures were constant, black root rot was less injurious in its retarding action on the growth of older plants than on the growth of younger plants. High soil temperatures were not so effective in protecting seedlings against retardation of growth due to black root rot as were these same high temperatures when the plants became older.

THIRD SERIES

In the third series of experiments, soil reactions were so adjusted as to give the following pH values: 5.5, 5.6, 5.7, 5.8, 5.9, and 6.0. The soil temperatures used were the same except that 32° C. was substituted for the 30° C. of the previous series.

[1] Expressed as relative numbers with dry weights at pH 5.6 as base.

TABLE 3.—*Effect of soil reaction and temperature on black root rot and on growth of tobacco*

[Third series]

Soil pH value	Extent of root rot on tobacco plants of different sizes when grown in infected soil at a temperature of—											
	15° C.		18° C.		21° C.		24° C.		27° C.		32° C.	
	Relative dry wieght of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot	Relative dry weight of plants [1]	Black root rot
5.5	98	None	103	None	96	None	110	None	87	None	91	None
5.6	100	Trace	100	do	100	do	100	do	100	do	100	Do.
	[2] 2.23		[2] 3.83		[2] 4.61		[2] 5.00		[2] 6.54		[2] 8.02	
5.7	67	Moderate	74	Mild	105	Trace	112	Trace	102	Trace	103	Do.
5.8	59	Severe	53	Severe	82	Moderate	63	Moderate	70	Moderate	106	Do.
5.9	50	do	58	do	56	Severe	43	Severe	63	do	100	Do.
6.0	59	do	55	do	57	do	56	do	62	do	107	Do.

[1] Expressed as relative numbers with dry weights at pH 5.6 as base.
[2] Weight expressed in grams.

The degree of root infection and the average dry weights of plants in each treatment, the latter expressed as relative numbers, are recorded in Table 3.

No more than a trace of infection by *Thielavia basicola* was present in soil with a pH value of 5.5 or 5.6 at any soil temperature. There was infection in soils with pH values of 5.7, 5.8, 5.9, and 6.0, and the critical point for black root rot on the pH scale was affected somewhat by the soil temperatures. At 15° C. growth was reduced about one-third and infection was of moderate severity at pH 5.7. At 18° C. black root rot was mild and growth was reduced about one-fourth at pH 5.7, and at pH 5.8 the disease was severe and growth was depressed by half. At 21° and 24° C., black root rot did not cause injury at pH 5.7, but at pH 5.8 infection was moderately severe and growth was somewhat retarded, and at pH 5.9 infection was severe and growth was retarded by about one-half. At 27° C. infection was only moderate in any soil, but there was a growth depression beginning at pH 5.8 or 5.9. At 32° C. there was no infection and no interference with growth even in soil with a pH value of 6.0.

In this series, as in the previous one, the critical region for black root rot on the pH scale was between pH 5.6 and 5.9. In the coldest soil, 15° C., the critical point was pH 5.7. At 18° it was pH 5.7 or 5.8; at 21° and 24° C. it was pH 5.8; and at 27° it was pH 5.8 or 5.9. Above 27° C. black root rot did no damage.

The indications are that tobacco in this soil would be safe from black root rot at any temperature at which this crop will grow in soil with a pH value of 5.6. Only in a very cold season would black root rot cause loss in this soil when the pH value was 5.7. If this soil had a pH value of 5.8 or 5.9, black root rot would be expected to cause growth depression below soil temperatures of 27° C. At 32° C., this soil would be safe with a pH value at least as high as 6.0.

But one would probably not be justified in attempting to specify definitely for all soils the pH value at which black root rot might occur, for the disease is known to be affected somewhat by the temperature of the soil, and it is not unlikely that it is also affected somewhat by the nature of the soil itself.

RELATION OF BLACK ROOT ROT TO THE TEMPERATURE AND REACTION OF SOIL IN THE FIELD IN 1927 AND 1928

The observations previously recorded were, of course, based on constant soil temperatures, but in the field during the growing season of tobacco the soil temperature is far from constant.

To supplement information secured in constant-temperature tanks, there is always, as has been pointed out by others (*12*), need for observations on the correlation of soil temperatures and plant diseases in the field. While a record of air temperatures is of some value, at least in comparing one growing season with another, a record of actual soil temperatures in the field is highly desirable, for, as shown by Keen and Russell (*13*) and as was suggested earlier by the work of Stockbridge (*19*), organisms in the soil are living in a rather warmer environment than would be inferred from the air temperatures.

A record of soil temperatures, 5 inches deep, was obtained in July and August, 1927 and 1928, in a tobacco field at Amherst, Mass. The daily maximum and minimum and the hourly mean temperatures of this soil are given graphically in Figures 1 and 2.

For the present, it is probably safer to consider the hourly mean temperatures rather than the daily maximum or minimum tempera-

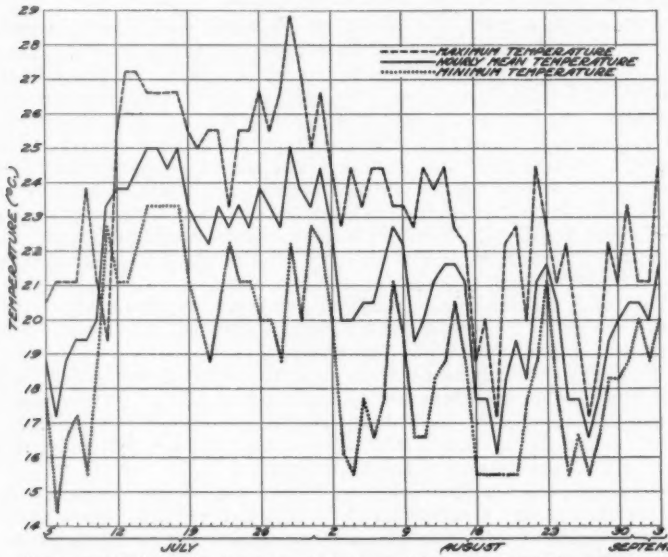

FIGURE 1.—Maximum, hourly mean, and minimum soil temperatures in field, summer of 1927

tures, although as has been pointed out by other investigators (*12*), the relative influence on plant diseases of daily maximum or minimum

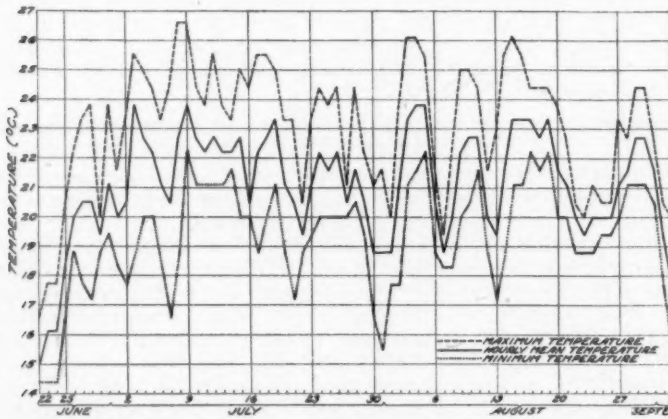

FIGURE 2.—Maximum, hourly mean, and minimum soil temperatures in field, summer of 1928

temperatures as compared with hourly mean temperatures is not known.

The pH values of the soil in this field during the growing seasons of 1927 and 1928 were 5.9 to 6.1. On the basis of results secured in

the temperature tanks and previously described, marked injury from black root rot would be expected in soil with these pH values if the soil temperatures were 24° C. or lower.

In 1927 the hourly mean temperatures of the soil in this field were below 24° C., and therefore low enough to favor infection by Thielavia from July 5 to September 3, with the exception of six days in July and one in August.

Under these conditions of soil temperature and reaction, root infection was severe. The yield per acre of cured leaf was only 987 pounds, while in adjacent plots the yield per acre was 1,659 pounds. In these plots the soil temperature was presumably the same as in the field, but the pH value of the soil was 5.6, too acid for black root rot.

In 1928 the hourly mean temperatures of the soil were below 24° C. from June 22 to September 2. As was to be expected, considerable black root rot developed, although it was not so severe as in 1927 in the same plots, probably because one more year had elapsed since this soil was limed (in 1923).

In 1927 the soil temperatures in the field were probably about normal in July and lower than normal in August, for the mean hourly air temperature at Amherst was 0.22° C. below normal in July and 1.98° C. below normal in August of that year. In 1928, however, the mean hourly air temperatures were not below normal at Amherst in either July or August. July and August in 1928, but not in 1927, were characterized by a total precipitation markedly greater than normal. So in these two years—one of slightly subnormal air temperature and approximately normal rainfall, the other of normal air temperature but excessive rainfall—the mean hourly temperatures of the soil were low enough (below 24° C.) to favor black root rot in this soil with a pH value of about 6.0.

EFFECT OF SOIL TEMPERATURE AND REACTION ON GROWTH OF PLANTS IN SOIL NOT INFESTED WITH THIELAVIA BASICOLA

In the experiments previously described, the higher the soil temperatures up to 27° to 30° C., the larger were the tobacco plants, and this was true in the practical absence of infection, that is in the more acid soils, as well as in the less acid soils on which there was black root rot. The optimum temperatures for the growth of tobacco (between 26° and 30° C. (*12*)) were, in the present experiments, about the same whether or not black root rot was present. Because of this, the condition of the roots, as well as growth measurements, was considered in determining the effect of soil temperature on black root rot.

The effect of soil reaction on the growth of tobacco in the absence of *Thielavia basicola* deserves consideration if we are to distinguish between soil reaction and the fungus itself as a cause of the poor growth of tobacco in soils with relatively high pH values. It is conceivable that the roots of tobacco plants might be more susceptible to infection if, in soil with a reaction approaching neutrality, the plants were weakened or their growth retarded by the too high pH value of the soil. But, according to the literature, the application of lime and the resulting higher pH value of the soil would benefit tobacco, at least in most soils of the Connecticut Valley, were it not for the presence of the black root-rot fungus. In the experiments of

Shchepkina (*20*) the optimum pH value for the growth of tobacco in solutions was 8.0. Briggs (*4*), working with a Connecticut Valley soil not infested with the fungus, found that tobacco made a better growth on limed than on unlimed soil. Chapman (*6*) concluded from his observations and experiments that liming is needed for the best growth of tobacco in Massachusetts. The immediate effect of liming an acid soil at this station was found by Anderson, Osmun, and Doran (*3*) to be an increase in the yield of tobacco, and not until a later year when black root rot developed were yields lowered. Morgan and Anderson (*14*) applied lime to an acid soil in Connecticut, and a marked increase in the yield of tobacco resulted.

It is of course possible to overdo the liming, and the unfavorable results on tobacco in such cases have been attributed, and with good cause, to the black root rot which follows. But evidently too much lime may be applied even when black root rot is not a factor. Johnson and Hartman (*10*) found that even in soil not infested with *Thielavia basicola* some lime increased the growth of tobacco, but that if more lime were added growth was retarded, at least at first.

In experiments by Slagg and his associates (*18*), the application of lime for three successive years was followed by lowered yields of tobacco, and meanwhile there was an increase in black root rot on limed plots; so the lowered yields might be attributed directly to the disease and only indirectly to the lime.

In order to secure further information on the effect of lime on tobacco, leaving out for the time the question of black root rot, the experiments described below were conducted.

EFFECT OF LIME ON STRAINS OF TOBACCO SUSCEPTIBLE TO BLACK ROOT ROT COMPARED WITH ITS EFFECT ON RESISTANT STRAINS

If a too high pH value of soil rather than increased infection by *Thielavia basicola* were the direct cause of trouble with tobacco in certain soils, as great a depression in yield would be expected in the case of strains of tobacco resistant to black root rot as in the case of susceptible strains in limed soils. That such is not the case is shown by the following results.

Havana tobacco of a strain susceptible to black root rot and Havana tobacco of two resistant strains[4] were set in adjoining plots in a field known to be infested with *Thielavia basicola*. In the case of each strain, half the plots used had been limed heavily (a total of 5 tons per acre, the last of it applied three years previously), and these plots had a pH value of 6.0. The other half of the plots used had not been limed, and these had a pH value of 5.4.

Roots were examined at the end of the season. There was only a trace of infection in the more acid soil on any strain, susceptible or resistant. In the less acid soil, black root rot was severe on the susceptible strain and very mild or present only as a trace on the resistant strains.

The yields per acre of cured leaf and the percentage loss in yield of limed plots, as compared with the yield of unlimed plots, are recorded in Table 4, together with pH values of soil and degree of infection by *Thielavia*.

[4] Developed by James Johnson of the Wisconsin Agricultural Experiment Station.

TABLE 4.—*Effect of liming the soil on the growth of strains of tobacco resistant and susceptible to black root rot*

Strain of tobacco	Grown without lime			Grown with lime			Percentage loss in yields on limed plots compared with yield on unlimed plots
	Soil pH value	Extent of root rot	Yield of tobacco per acre	Soil pH value	Extent of root rot	Yield of tobacco per acre	
			Pounds			*Pounds*	
Susceptible	5.4	Trace	1,294	6.0	Severe	821	37
Resistant, No. 142A3X	5.4	do	2,255	6.0	Trace	2,222	1
Resistant, No. 142C3X	5.4	do	2,176	6.0	do	2,049	6

The yield of the susceptible strain, with black root rot a factor, was 37 per cent less on limed than on unlimed plots. The yields of the resistant strains, with black root rot not a factor, were only 1 per cent and 6 per cent less on limed than on unlimed plots. In other words, when black root rot was not a factor, there was no real or significant decrease in yield as a result of the lime.

EFFECT OF SOIL REACTION ON GROWTH OF TOBACCO IN ABSENCE OF THIELAVIA BASICOLA COMPARED WITH ITS EFFECT IN THE PRESENCE OF THIS FUNGUS

Soils known to be infested with *Thielavia basicola* were obtained from a limed plot in which the pH value of the soil was 6.0, and from an adjacent unlimed plot in which the pH value was 5.5.

Half the soil obtained from each plot was steam sterlized to rid it of the fungus. As has been pointed out by others (*10*), an absolute comparison between sterilized and infested soils is subject to the criticism that changes in the soil in addition to the eradication of parasitic fungi result from steam sterlization. But methods of disinfecting soil without otherwise altering it are unknown, and for the purposes of this experiment, soil entirely free from *Thielavia basicola* was needed.

Five weeks after the soils were sterilized, tobacco seedlings (from sterilized soil) were set in pots of the several soils, with treatments in triplicate. The average dry weights per plant in each treatment at the end of the experiment are recorded in Table 5.

TABLE 5.—*Effect of liming the soil on the growth of tobacco with and without Thielavia basicola in the soil*

Description of soil	Average dry weight per plant grown
	Grams
Limed, Thielavia present	2.46
Not limed, Thielavia present	3.19
Limed, Thielavia absent	12.18
Not limed, Thielavia absent	3.45

Roots were examined. Infection by *Thielavia basicola* was severe in limed soil not sterilized, very mild or little more than a trace in soil not limed and not sterilized, and absent from sterilized soils limed and not limed.

When neither the limed nor the unlimed soil was sterilized, infection by the fungus in the limed soil so retarded the growth that plants in it had an average dry weight only 77 per cent of that of plants in unlimed soil.

When both these soils were sterilized, the benefit of lime was such that the average dry weight of plants in limed soils was 353 per cent of that of plants in soil without lime. In sterilized soil lime was associated with a marked increase rather than a decrease in the growth of tobacco plants.

In soil limed, and with the fungus consequently an important factor, the response in growth following the eradication of the fungus by steaming was an increase in weight of plants of 395 per cent. In soil not limed, and so without the fungus as an important factor, the response in growth following steaming was an increase in weight of plants of but 8 per cent, which is hardly significant. This may be interpreted to mean that a limed soil responded much more markedly to the stimulatory effect of steaming than did a soil without lime, but in any case it is evident that lime alone in the absence of *Thielavia basicola* did not injure the plants.

EFFECT OF SOIL TEMPERATURE ON THE RESPONSE OF TOBACCO TO CERTAIN SOIL REACTIONS IN THE ABSENCE OF THIELAVIA BASICOLA

The effect of soil reaction on the growth of tobacco in soil infested with *Thielavia basicola* is affected by the temperature of the soil. The experiments described below were undertaken to determine whether or not the response of tobacco to certain soil reactions is also affected by the temperature of the soil when this fungus is not a factor.

The soil used was freed of the fungus by steam sterilization. Its initial pH value was 5.6. The soil was divided into several portions and their reactions adjusted by means of hydrated lime, after preliminary experiments had shown the quantities of lime necessary to produce certain desired changes in the pH value of the soil. The quantities of lime used, on an acre basis, and the resulting pH values of the soil are shown in Table 6. This soil was placed in pots in constant temperature tanks, and one month after the application of lime, tobacco seedlings from sterilized soil were set in each pot.

TABLE 6.—*Effect of soil temperature on the response of tobacco plants to lime in the absence of Thielavia basicola*

Treatment of soil (per acre)	Soil pH value	Average dry weight (grams) per plant and percentage loss or gain in weight as a result of liming soil at a temperature of—											
		15° C.		18° C.		21° C.		24° C.		27° C.		30° C.	
		Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain	Weight	Loss or gain
No lime	5.6	1.83	------	4.33	------	5.17	------	4.69	------	4.49	------	4.08	------
2.75 tons lime	6.0	1.04	−43	3.11	−28	4.99	−3	3.44	−27	6.04	+35	5.97	+46
5.5 tons lime	6.3	1.05	−43	2.37	−45	2.76	−47	6.42	+37	7.17	+59	6.49	+59
7.0 tons lime	6.5	.96	−48	2.00	−54	2.90	−44	3.91	−17	4.19	−7	6.78	+66

The average dry weights per plant at the end of the experiment in each treatment at each soil temperature are recorded in Table 6 together with the percentage increase or decrease in dry weights of plants in each lime treatment as compared with the weight of plants without lime at the same temperature. The effects of the several treatments on growth of plants are also shown in Figure 3.

In the sterilized soil, with *Thielavia* absent, plants grew markedly better without lime than with lime at the lower soil temperatures, below 24° C.; but plants grew markedly better with lime than without lime at the higher soil temperatures, 27° and 30° C. (Table 6.)

After the plants in this series were removed, tobacco seedlings were again set in the soil without lime and in the soil which received lime at the rate of 2.75 tons per acre. At the end of this experiment, the dry weights of plants in the soil with lime were, as compared with the dry weights of plants in soil without lime, as follows: 54 per cent less at 15° C., 59 per cent less at 18° C., 5 per cent less at 21° C., 11 per cent less at 24° C., 39 per cent greater at 27° C., and 87 per cent greater at 30° C. As in the preceding experiment, lime at the higher soil temperatures resulted in plants larger and at the lower soil temperatures in plants smaller than the plants without lime at the same temperatures.

In these experiments, the effect of lime in soil without *Thielavia basicola* present

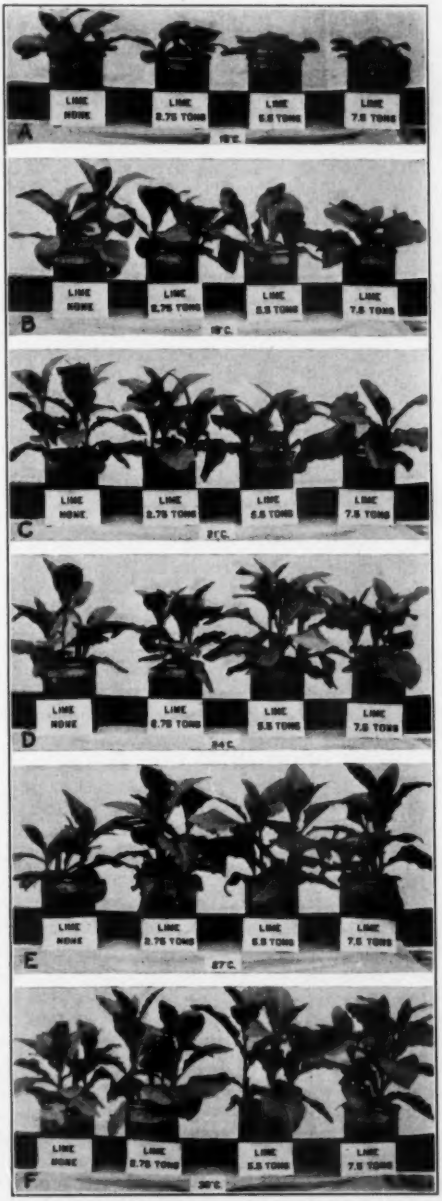

FIGURE 3.—Effect of soil temperature on the response of tobacco plants to lime in the absence of *Thielavia basicola*. Lime applications (per acre) and soil temperatures are indicated in the figure

was to increase growth of tobacco at soil temperatures optimum for its growth but to retard the growth of tobacco at lower temperatures. The effect of lime on tobacco in soil without this fungus was not unlike the effect which lime is known to exert on tobacco with *T. basicola* present. But if tobacco fails to grow at optimum soil temperatures in soils limed, even heavily limed, the cause is to be looked for in the blackroot-rot fungus rather than directly in the lime itself.

The response in the growth of tobacco to the beneficial effect of higher soil temperatures was greater with than without lime. (Table 6.) In this connection, results secured by Cerighelli (*5*) are of interest. He found that raising the temperature 10°, or to the optimum, increased the growth of roots of peas with calcium present but not without calcium.

No attempt is made to generalize from the results of these experiments, since the conditions were of course different from those in the field; the soil was steam sterilized, no nitrogenous fertilizers were applied, and relatively large amounts of lime were used to produce the desired changes in the pH value of the soil. It is not unlikely, however, that in the field, as in these pot experiments, an application of lime beneficial to tobacco at higher soil temperatures would retard the growth of this plant at lower soil temperatures. The explanation is probably to be found in the relation of lime to nitrification in the soil, for, although lime is favorable to nitrification, it is known that the decomposition of organic matter which results from liming may be accompanied by a loss of nitrogen from the soil if other conditions are unfavorable for the formation of nitrates, and one of these unfavorable conditions is known to be soil temperatures which are too low.

EFFECTS OF LIME, SOIL DISINFECTION, AND SOIL TEMPERATURE ON GROWTH OF TOBACCO PLANTS IN SEED BED

Since tobacco growers have learned the connection between lime and black root rot, few if any of them in this section now apply lime to the soil of the seed beds. It was probably applied more generally in the past, however, for Garner (*8*), describing in 1922 the culture of Connecticut Havana seed tobacco, mentions the application of lime, about 2 tons per acre, to tobacco seed beds (in the fall).

If lime benefits tobacco in the seed beds, it could of course be used without anticipating injury from black root rot in those seed beds in which the soil is sterilized. But the seed beds here are planted as early in the spring as the frost is out of the ground and when the soil temperatures are low. If the soil temperatures in the seed bed were for a long period low enough, injury rather than benefit from lime would be expected, even in sterilized soil, on the basis of the results of the experiments previously described in which lime interfered with the growth of tobacco at soil temperatures below 24° C., even in the absence of *Thielavia basicola*. The experiment now to be described was undertaken to secure further information as to the effect of lime on the growth of tobacco at known soil temperatures in the presence and in the absence of that fungus.

The soil in part of a seed bed was treated according to the standard method of soil disinfection with formaldehyde 1 to 50, 2 quarts per square foot being used. Two weeks later, one week before seeding, agricultural lime (50 per cent CaO) was applied at the rate of 2 tons

and of 4 tons per acre to plots in both the disinfected and the non-disinfected areas in this seed bed. Tobacco was seeded in all plots April 12, 1928.

By May 16, plants in formaldehyde-treated soil were larger than plants in soil without formaldehyde, but there was then no visible effect of lime. By May 31 the effect of lime on growth was evident. There was a conspicuous lag in the growth of plants on the formaldehyde-treated plot to which lime had been applied. The average dry weight of 100 typical plants removed from each plot on this date are recorded in Table 7, and the relative sizes of the plants at this time are shown in Figure 4.

TABLE 7.—*Effect of soil reaction on growth of tobacco in seed bed at known temperatures, with and without soil disinfection*

Treatment of soil (per acre)	Resulting pH value of soil [1]	Size of plants, May 31		Size of plants, June 14		Extent of black root rot
		Average dry weight	Relative weight [2]	Average dry weight	Relative weight [2]	
		Grams		*Grams*		
No disinfection; no lime	6.01	1.57	100	8.70	100	Moderate.
No disinfection; 2 tons lime	7.06	1.26	78	6.80	79	Severe.
Formaldehyde; no lime	5.96	5.53	348	6.10	70	Trace.
Formaldehyde; 4 tons lime	6.90	2.14	136	5.40	62	Mild.
Formaldehyde; 2 tons lime	7.00	2.50	159	5.60	64	Do.

[1] Determined electrometrically, May 12, by means of Youden hydrogen-ion concentration apparatus.
[2] Calculated with average dry weight of plants grown in unlimed, undisinfected soil as a base.

In the absence of lime, plants were more than three times as large in the formaldehyde-treated plot as in the plot without formaldehyde. In soil to which formaldehyde was not applied, plants were smaller with lime than without lime (relative numbers 78 and 100, respectively). But the harmful effect of lime was relatively much greater in soil disinfected with formaldehyde than in soil not so treated. In soil treated with formaldehyde the application of lime (2 tons per acre) resulted in plants less than half the size of plants without lime (dry weights expresses as relative numbers, 159 and 348, respectively).

On May 31 plants from the plot treated with formaldehyde but with no lime were large enough to transplant to the field; but plants from the plots not treated with formaldehyde or plants from the plots which received both formaldehyde and lime did not become large enough to transplant until two weeks later.

The benefit of formaldehyde, either in inhibiting parasites or in directly stimulating plant growth was marked up to the end of May, after which, with soil temperatures, especially the daily minima, higher, plants without formaldehyde grew relatively more rapidly. Plants were again removed from each plot two weeks later (on June 14), air-dried, and weighed. The weights are recorded in Table 7. The increases in dry weights at this time, as compared with the dry weights of the plants in each treatment two weeks earlier, were as follows: No disinfection, no lime, 454 per cent; no disinfection, 2 tons lime, 439 per cent; formaldehyde, no lime, 10 per cent; formaldehyde, 2 tons lime, 124 per cent; formaldehyde, 4 tons lime, 152 per cent.

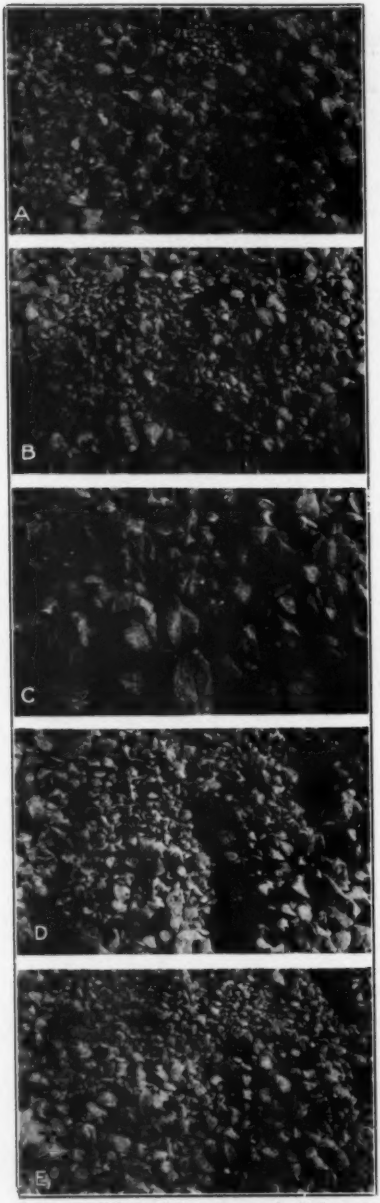

FIGURE 4.—Effects of lime on growth of tobacco in seed bed with and without soil disinfection with formaldehyde: A, No disinfection, no lime; B, no disinfection, 2 tons lime per acre; C, formaldehyde treatment, no lime; D, formaldehyde treatment, 4 tons lime per acre; E, formaldehyde treatment, 2 tons lime per acre

These differences resulted partly from the fact that the relatively rapid growth characteristic of tobacco plants at a certain age had already been made two weeks earlier by the plants in the formaldehyde-treated plot without lime.

Roots were examined June 14. In the plots without formaldehyde treatment, black root rot was a real factor; the disease was severe with lime and at least moderate without lime. There was some black root rot in all plots, even in those in which the soil received the formaldehyde treatment. However, there was no more than a trace of black root rot on plants in the plot with formaldehyde and no lime, and the disease was mild in formaldehyde-treated plots with lime. The formaldehyde was applied early in the spring, as is the usual practice at this station, and the soil was more or less saturated with water and packed. Under these conditions it is unlikely that formaldehyde treatment ever results in the complete absence of black root rot in a seed bed if the soil was infested before treatment.

A recording thermometer was placed in the seed bed and the temperature of the soil, at a depth of 2 inches, was thereby determined, beginning April 12. The daily maximum, minimum, and hourly mean temperatures so obtained are graphically expressed in Figure 5.

From April 12 to June 3, the hourly mean temperatures of the soil in the seed bed were always below 24° C., and the daily maximum temperatures of the soil were often below that point. The hourly mean temperatures of the soil in April were lower than they were later in the spring, although in this glass-covered seed bed the maximum daily temperatures were in some cases above 24° C. in April. But the daily ranges between maximum and minimum soil temperatures were greatest in April

because of the very low minimum soil temperatures in that month usually between 7° and 9° C.

This is the record in a cold and backward spring. (The mean hourly temperatures of the air were 2° C. below normal in April and 1.4° C. below normal in May at Amherst in 1928.)

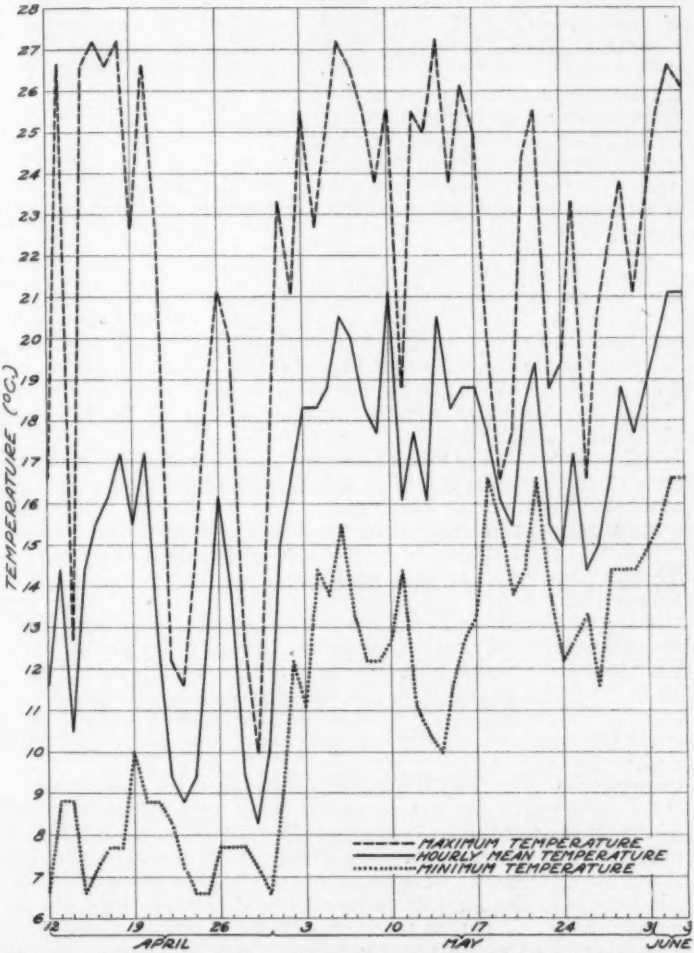

FIGURE 5.—Maximum, hourly mean, and minimum soil temperatures in tobacco seed bed, 1928

Under these conditions of low soil temperatures, the application of lime to the soil in the seed bed retarded the growth of tobacco, as it had in the pot experiments previously described, even when black root rot infection was reduced to no more than a trace by the use of formaldehyde. (It should be noted, however, that the disinfecting

effect of formaldehyde was not sufficiently complete to prevent some increase in black root rot when lime was applied to a formaldehyde-treated plot. It should further be noted that this soil was in no apparent need of lime for tobacco in the beginning, since it had an initial pH value of 5.96 to 6.01.)

But the evidence is unfavorable to the application of lime to a tobacco seed bed, at least in the spring, even though the soil has practically been rid of *Thielavia basicola* by formaldehyde, for in these experiments tobacco plants so treated were two weeks later in reaching transplanting size than were plants without lime, with the soil temperatures usually below 24° C.

In another experiment,[5] lime was applied in the spring to the soil of a seed bed not sterilized. Growth of tobacco plants in this seed bed was not affected by applications of lime up to 1,600 pounds per acre. There was no black root rot on any of the plants, but growth was retarded by applications of lime at the rate of 2,000 pounds or more per acre.

SUMMARY

The critical point for black root rot of tobacco on the pH scale was affected somewhat by soil temperature. In the soil used there was no black root rot or only a trace at any temperature in soil with a pH value of 5.6 or lower. Marked injury began at pH 5.7 at 15° C., at pH 5.7 or 5.8 at 18° C., at pH 5.8 at 21° and 24° C., and at pH 5.8 or 5.9 at 27° C. There was little or no injury at 30° C., even in soil with pH values of 6.0 to 6.9.

Black root rot in the less acid soils at pH 5.9 to 6.5, with the soil temperature constant, retarded the growth of tobacco plants more when they were young than when they were older. Higher soil temperatures did not as effectively protect seedlings as they did older plants against retardation of growth caused by black root rot.

The germination of tobacco seeds was not affected by soil reaction within a range of soil pH values of 4.6 to 6.6. Soil temperatures between 15° and 30° C. had less effect on the percentage of seeds germinating than on the length of time required for germination.

The incubation period for infection of tobacco seedlings by *Thielavia basicola* was two to three weeks under optimum conditions. The colder the soil, down to 18° C., and the more nearly neutral the soil reaction (as compared to more acid soils) the shorter was the incubation period.

In the growing seasons of 1927 and 1928, the first characterized by slightly subnormal air temperatures with approximately normal precipitation and the second by normal air temperatures with excessive precipitation, the mean hourly temperatures of the soil in a tobacco field at Amherst, Mass., were below 24° C., and therefore favorable to black root rot in this soil (pH value about 6.0), with the exception of a few days in 1927. Under these conditions of soil temperature and soil reaction black root rot was sufficiently severe to reduce the yield of tobacco 40 per cent in 1927, as compared with the yield in the more acid soil of adjoining plots.

[5] This experiment was conducted by J. P. Jones, of the Massachusetts station, who has kindly allowed the writer to use this material, not previously published.

In a soil heavily limed and consequently infested with *Thielavia basicola*, the yield of a strain of Havana tobacco susceptible to black root rot was 37 per cent less on limed plots than on unlimed plots, but the yield of strains resistant to black root rot was not significantly less on the limed plots than on the plots not limed, indicating that it is this fungus and not lime which is the direct cause of the poor growth.

In the absence of *Thielavia basicola*, the application of lime to a soil did not interfere with the growth of tobacco; on the contrary, it increased it.

The response in the growth of tobacco to certain soil reactions was affected by the temperature of the soil, even though *Thielavia basicola* was first eradicated by soil sterilization. Applications of lime which benefited tobacco at higher soil temperatures (27° and 30° C.) resulted in a retardation in its growth at lower soil temperatures (below 24° C.) in both cases as compared with the growth of tobacco without lime at the same temperatures.

In a glass-covered tobacco seed bed the hourly mean temperatures of the soil were below 24° C. from April 12 to June 3. Under these conditions the application of lime (by which the pH value of the soil was changed from 5.96 or 6.01 to 6.90 or 7.00) seriously retarded the growth of tobacco, even in soil in which black root-rot infection had been reduced to no more than a trace by the earlier formaldehyde treatment of the soil. The harmful effect of lime in the seed bed was relatively much greater in soil disinfected with formaldehyde than in soil not so treated.

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DEFICIENCY OF MAGNESIUM THE CAUSE OF A CHLOROSIS IN CORN[1]

By J. P. JONES

Research Professor of Agronomy, Massachusetts Agricultural Experiment Station

INTRODUCTION

Many investigators have studied the chlorosis of plants and have found it to be induced by a number of conditions. If chlorosis, as defined by Clinton (*3*),[2] is "that unusual state of a green plant in which the chlorophyll, through either diseased or limited development, does not possess its normal bright green color, but becomes lighter, yellowish-green, or even distinctly yellowish or whitish," the types of chlorosis may be classified as follows: (1) Infectious chlorosis, such as the mosaics; (2) hereditary chlorosis, as shown in variegated plants; and (3) nutritional chlorosis, or a failure in the normal development of chlorophyll attributable to a specific nutrient deficiency or to a toxin.

The chlorosis with which this paper is concerned occurred on corn in one of the experimental fields at the Massachusetts Agricultural Experiment Station, known as North Corn Acre, and was first observed about 1920. It had probably been present prior to that time but in a less severe form, and so escaped attention. It was not until 1924 that definite work to determine the cause of chlorosis was undertaken. Observations up to this time showed (1) that no infectious disease was apparent, and (2) that the corn grown was inherently normal in color. It therefore appeared likely that the chlorosis was of the nutritional type. When the work was begun it was designed to attack the problem from the nutritional standpoint. This paper reports the results obtained.

DESCRIPTION OF THE CHLOROSIS

The symptoms of the chlorosis noted on North Corn Acre were manifested early in the season by a striping of the leaf. The striping was due to a differentiation in color of the intervascular and vascular tissues. About two weeks after the corn came up the intervascular tissue showed a light green color while the vascular tissue was a deep green, almost normal color. As the season progressed the light green color of the intervascular tissue continued to fade until all signs of green disappeared. Before the complete disappearance of the green color necrosis set in and the tissue turned yellow to yellowish brown and gradually ceased to function as a living part of the plant. The vascular tissues persisted in maintaining a green color almost until the adjacent tissues were dead, although evidences of fading appeared somewhat sooner. In extreme cases both the vascular and the intervascular tissues, particularly those near the border and tip of the leaf, lost their green color, turned yellowish

[1] Received for publication Apr. 11, 1929; issued December, 1929.

[2] Reference is made by number (italic) to "Literature cited," p. 891.

brown, and dried up prematurely. Illustrations of chlorosis at different stages may be seen in Figure 1.

Another symptom frequently noted was the bronzing and reddening of the leaves. In the early season this was observed chiefly near the margins but later it seemed to disappear. In the late

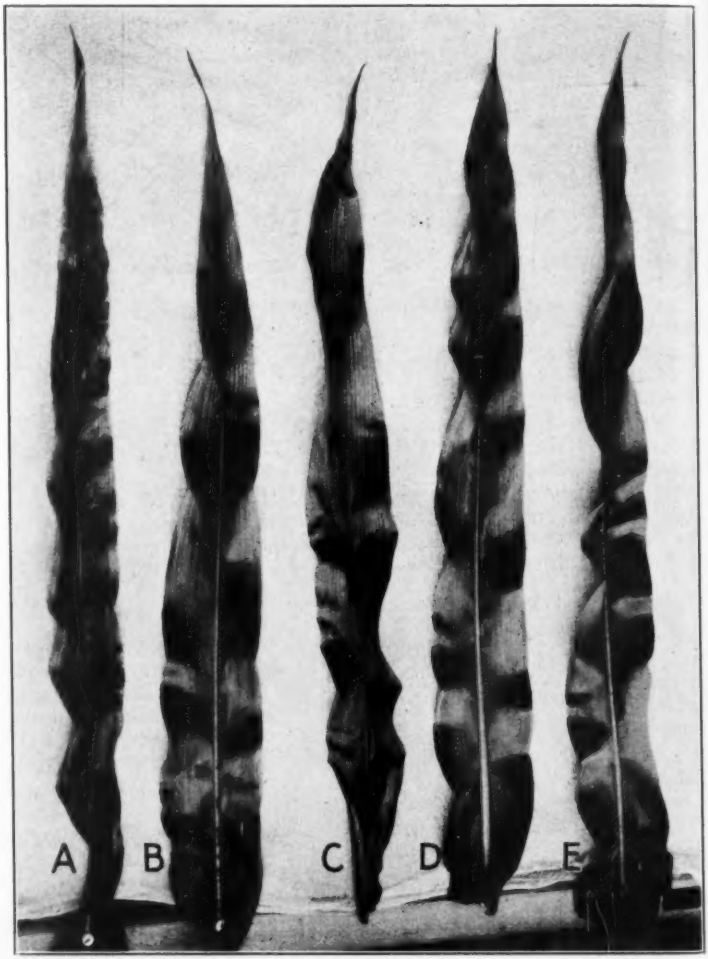

FIGURE 1.—Variation in degree of chlorosis on different corn leaves: A, Extreme chlorosis; B and C, medium chlorosis; D, slight chlorosis; E, normal green leaf

season, however, when the leaves began to mature, many deep-red leaves were observed. Whether this is a symptom closely associated with the chlorosis in question has not been definitely determined.

The chlorosis varied in severity among different plants and among leaves on the same plant. The bottom leaves seemed to be affected

most severely and the upper leaves only slightly. The tips and borders of the leaves were generally attacked most severely. The yellowing of the intervascular tissues in the early stages could usually be traced from the tip to the base of the leaf blades. The more severe aspects of the chlorosis, such as browning and dying of the tissue, were usually observed first in the regions near the tip and border of the leaf. It was also not uncommon to find plants exhibiting rather extreme cases of chlorosis growing within 3 feet of plants of a normal green color. The proportion of normal plants in a given area seemed to be greatest where the leaves showed only slight injury from chlorosis.

Whether the growth of the corn plant was affected seemed to depend upon the severity of the chlorosis. In light cases no visible differences in rate of growth were noticeable, but in extreme cases growth was reduced. The reduced growth was not especially marked early in the season, but in the latter part of the season the chlorotic corn grew somewhat more slowly and matured earlier. Even in cases where chlorosis was slight earlier maturity was characteristic.

CONDITIONS UNDER WHICH CHLOROSIS DEVELOPED

The field on which chlorosis developed had been used for fertilizer tests since 1891. Thus the conditions under which the chlorosis of the corn was brought about can be definitely described from the records.

The soil of North Corn Acre is a fine sandy loam underlaid by a gravelly fine sand which varies in depth from 12 to more than 40 inches. The field is well drained and has been classified as belonging to the Merrimac series. The topography is rolling, with a gently sloping ridge running diagonally from approximately northeast to southwest across the whole field. On this part a considerable amount of gravel is found in the surface soil.

During the period from 1891 to 1920, inclusive, two types of fertilizer for corn were being compared. One, commonly known as "special corn" fertilizer, was relatively high in phosphoric acid and low in potash; the other was relatively low in phosphoric acid and high in potash. For the purpose of this experiment North Corn Acre was divided into four plots, each one-fourth acre in size. Plots 1 and 3 received the special corn fertilizer and plots 2 and 4 received the fertilizer high in potash. The average annual application (1891 to 1920, inclusive) of nutrients furnished by the two fertilizers is shown in Table 1. Plot 4 received the same treatment as plot 2, except that from 1907 to 1918 inclusive an addition of 400 pounds of basic slag was applied annually, which accounts for the high average of P_2O_5 shown in Table 1 for plot 4.

The materials used to furnish the nutrients varied from time to time, the reason probably being the changes in the market supply that would normally occur during so long a period. As a source of nitrogen, nitrate of soda was used from 1891 to 1895 inclusive; and, with the exception of a small amount of sulphate of ammonia used in 1919 and 1920, nitrate of soda, dried blood, and fish were employed from 1896 to 1920 inclusive. The phosphoric acid was furnished by dissolved bone black from 1891 to 1895 inclusive, and by superphosphate (acid phosphate) and fish from 1896 to 1920 inclusive. Except in 1919

and 1920, when Nebraska potash was used, muriate of potash furnished the potash for the entire period from 1891 to 1920 inclusive. In spite of these variations in materials, the original purpose of comparing the two types of fertilizer was adhered to until 1921.

TABLE 1.—*Quantity of nutrients and fertilizers applied annually to the different plots in North Corn Acre from 1891 to 1928, inclusive*

Plot No.	Nutrients applied 1891 to 1920, inclusive, expressed in average pounds per acre			Fertilizers applied 1921 to 1928 expressed in average pounds per acre			
	NH_3	P_2O_5	K_2O	Nitrate of soda	Fish	Superphosphate (acid phosphate)	Muriate of potash
1	50	156	58	120	360	----------	150
2	50	56	120	200	200	200	250
3	50	156	58	120	360	1,092	150
4	50	81	120	200	200	200	----------

Except in 1891 and 1892, when millet was planted on plots 3 and 4, corn was grown on all plots from 1891 to 1896, inclusive. In 1897 the cropping system was changed to two successive years of hay—a mixture of timothy, redtop, and clover—and then two successive years of corn. The hay mixture was seeded the second year in the corn. This cropping system has been used without change up to the present time.

No manure was applied during the entire period of the tests. Lime was used in 1900 at the rate of 1 ton per acre of air-slaked lime, in 1907 at the rate of 1 ton per acre of agricultural lime, and in 1921 at the rate of 2 tons per acre of ground limestone, but only on the north half of the plots.

The comparison between the two types of fertilizer revealed very little difference in response as measured by the yields of corn and hay. The average yields per acre of the crops grown up to 1921 were, for plots 1 and 3, 63.88 bushels of corn and 3,993 pounds of hay; for plots 2 and 4, 62.20 bushels of corn and 4,229 pounds of hay. These yields are based on field weights rather than on a dry basis. In view of the variability of the data, little significance can be attached to these differences in yields. Part of this variability is believed to have been due to the manner in which variation in rainfall affects the different plots. During a dry season crops on the section of the plots through which the ridge runs suffer severely, the different plots being unequally affected. Probably in some seasons the environmental variation was of greater significance than the difference in treatments.

HYPOTHESES TO ACCOUNT FOR DEVELOPMENT OF CHLOROSIS

In considering the cause of the chlorosis from the nutritional standpoint, two factors seemed to deserve first consideration: (1) The presence in the soil of an excess of soluble aluminum and (2) the lack of magnesium.

ALUMINUM TOXICITY

Preliminary observations showed that the corn growing on the half of the field limed in 1921 was less severely affected by chlorosis than that on the unlimed half. This pointed definitely to the con-

clusion that lime was capable of overcoming chlorosis, and furnished the basis for suspecting that aluminum toxicity was involved.

When Abbott, Connor, and Smalley (*1*) first noted poor crop yields associated with an excess of soluble aluminum salts they found applications of pulverized limestone under field conditions to be an effective remedy. The later work of Hartwell and Pember (*5*), Mirasol (*11*), and Burgess and Pember (*2*) showed superphosphate to have a capacity similar to that of lime in counteracting the depressing effects of aluminum. Their work indicated that for practical purposes moderate applications of lime and superphosphate were more effective than either alone in correcting the acidity of soils containing large amounts of active aluminum. If aluminum were a factor in causing the chlorosis on North Corn Acre, these results suggest that the chlorosis should have been less severe on plots 1 and 3, which received the high applications of superphosphate. (Table 1.) Preliminary observations, however, did not indicate any lessening of the chlorosis associated with the high superphosphate treatments.

Burgess and Pember (*2*) have classified certain crop plants according to their resistance to aluminum toxicity. In their classification corn is rated as very resistant, being capable of normal growth on soil having a pH value below 5.3 and an active aluminum content above 500 parts per million of dry soil. Magistad (*10*), working with nutrient solutions, showed that toxicity of aluminum to the corn plant varied with the pH, at pH 4 the toxic effect was very severe, while at pH 6.4 it was slight. The preliminary field observations indicated that the chlorosis appeared on soil having a pH value between 5 and 5.4.

Hoffer (*6*) has reported that aluminum salts are especially effective in plugging the vascular bundles of the corn plant. He also described the manner in which aluminum affected the foliage. The leaves first showed a slight yellowing of the intervascular tissue, resulting in a yellow streaked appearance, then a water-soaked effect, and finally in necrosis. These observations are quite similar to those recorded for the corn leaves on North Corn Acre. However, there was no evidence that the nodal tissue of the corn was destroyed as Hoffer (*6*) observed in cases of severe aluminum injury.

MAGNESIUM DEFICIENCY

Magnesium, being a constituent of chlorophyll and necessary for normal green color in plants, was suspected of being deficient in the soil on North Corn Acre and therefore a possible cause of the chlorosis. Garner and his associates (*4*) found that sand drown, a chlorosis of tobacco, resulted from a deficiency of magnesium in certain soils of the South and of the Connecticut Valley. They stated that applications of cottonseed meal, barnyard manure, tobacco stalks, and tobacco stems were effective in controlling sand drown. The effectiveness of these materials was explained as due to the magnesium which they contained. As mentioned above, the fertilizers used on North Corn Acre were practically free of magnesium. No barnyard manure or vegetable organic fertilizer has been applied in 37 years. The finding of sand drown on tobacco in the Connecticut Valley, in spite of the fact that it has been the practice to fertilize to a limited extent with barnyard manure and vegetable organic materials for many years, strongly supported the suspicion

that a magnesium deficiency existed on North Corn Acre. This evidence seemed particularly suggestive when it was recognized that the soil on this field is quite similar to that on which much of the tobacco is grown.

On North Corn Acre, as noted above, lime was effective in reducing chlorosis. The records showed that the lime used contained about 5 per cent magnesium oxide. Garner and his coworkers claim that liming the soil even with fairly pure limestone should prevent the sand-drown type of chlorosis. In field experiments in North Carolina (*8*) the yield and quality of tobacco have been improved by treating the soil with magnesian limestone.

METHOD OF PROCEDURE

The method of procedure was developed to determine whether lime prevented chlorosis through counteracting soluble aluminum or through furnishing needed magnesium. Field work was supplemented by greenhouse and chemical studies.

FIELD STUDIES

RESIDUAL VALUE OF PHOSPHORIC ACID AND POTASH IN THE SOIL

In 1921 the old objective of the North Corn Acre experiment—to compare two different fertilizers for corn—was abandoned. In its place was substituted an attempt to observe the residual value of the previous high applications of phosphoric acid and potash. This was done by omitting superphosphate from the fertilizer applied to plot 1. The residual influence of the superphosphate was expected to be shown by a comparison of plot 1 with plot 3, on which the original high applications of superphosphate were continued. In like manner potash was omitted from the fertilizer treatment on plot 4 with the expectation that a comparison with the continued high applications on plot 2 would show the residual effects of the many years of high potash treatment. The annual fertilizer applications from 1921 to 1928, inclusive, are shown in Table 1.

In Table 2 have been included data bearing on the residual value of the long treatment with both high potash and superphosphate. No yield records are given for 1928 because wireworm injury on certain areas so affected the yields that they can not be considered to represent the effects of the treatments reliably. The records for hay were also omitted because hay was grown in 1921 and 1922 immediately after the omission of superphosphate and potash from the treatments of plots 1 and 4, and no evidence of a shortage of these fertilizers was noted. In 1925 and 1926, when hay again occupied the field, the dry weather during the growing season affected the growth to such an extent that no yield records were taken.

It is apparent from Table 2 that the omission of the superphosphate from the fertilizer treatment on plot 1 did not affect the yield of either the corn or the stover. This may be taken to indicate that phosphoric acid (P_2O_5) applied at the rate of about 156 pounds per acre annually for 30 years in a rotation of corn and hay builds up a substantial residuum in the soil. On this field it was sufficient to maintain the yield of corn and stover for at least 7 years.

On the other hand, Table 2 reveals that an application of 120 pounds of potash (K_2O) per acre for 30 years did not permit a very large accumulation. However, the accumulation was sufficient to maintain the yield of corn on plot 4 the third year after the potash was left out of the fertilizer application. In the fourth and seventh years plot 4 as compared with plot 2 where the original high potash treatment was continued showed a decline in yield of corn of about 13 per cent. No consistent decline in yield of stover was apparent.

TABLE 2.—*Residual effect of phosphorus and potash on the yield per acre of corn and stover*

[Dry weight basis]

Plot No.	Yield in 1923		Yield in 1924		Yield in 1927		Average yield	
	Corn	Stover	Corn	Stover	Corn	Stover	Corn	Stover
	Bushels	*Pounds*	*Bushels*	*Pounds*	*Bushels*	*Pounds*	*Bushels*	*Pounds*
1	35.0	2,366	34.6	1,785	40.2	2,026	36.6	2,059
3	36.9	2,092	33.2	2,184	43.2	1,921	37.8	2,066
2	30.3	2,389	31.1	1,653	36.8	1,861	32.7	1,968
4	32.5	2,044	27.1	1,738	32.1	1,681	30.6	1,821

EFFECT OF LIME AND MAGNESIUM ON THE DEVELOPMENT OF CHLOROSIS

Cross treatments of the plots on which the residual effects of phosphoric acid and potash were being studied were made to compare the influence of magnesium and lime on the chlorosis of corn. Figure 2 shows the plan of the field. The numbers employed refer to the plots as they were laid out in 1891 and the letters to the cross treatments. Magnesium sulphate was applied in the spring of 1924 and 1927 at the rate of 200 pounds per acre and in 1928 at the rate of 400 pounds per acre. This gave cross treatments as follows: Section A, 2 tons of ground limestone per acre applied in 1921; section B, 2 tons of ground limestone applied in 1921 plus magnesium sulphate at the rates noted above; section C, magnesium sulphate at the same rate as on section B; section D, check, no lime or magnesium sulphate.

To determine the effects of the cross treatments on chlorosis, counts were made of what were recognized in the field as normal hills and as slightly, medially, and extremely chlorotic hills. For the normal hills only those entirely free of the typical chlorosis were counted. The attempt to classify the chlorosis into slight, medium, and extreme cases was based largely upon the judgment of the observer, and the line of demarcation between these groups can not be considered as accurate as that between the normal and chlorotic groups. The classification does, however, afford a basis for estimating the severity of the chlorosis.

In Table 3 the observations for 1924, 1927, and 1928 are recorded. Considering first the results for 1924, it will be noted that practically no chlorosis occurred on sections A and B on any of the plots. Such as did occur was rated as slight and probably would not be noticed under practical conditions. It will be recalled that A and B both received lime in 1921 and B magnesium sulphate in 1924. These records are in accord with the preliminary observations which showed that chlorosis was not very apparent on the limed area.

On section C, which received the magnesium sulphate treatment, the proportion of normal hills was somewhat smaller than on the limed area. Except on plot 2, where 12.38 per cent of medially chlorotic hills was noted, the chlorosis was rated as slight. In comparison with section D, which received neither lime nor magnesium sulphate, the treatment on section C seems to have been very effective. This is manifested both by the greater proportion of normal hills on each plot and by the milder form of the chlorosis.

The magnesium sulphate treatment was made about 10 days before the corn was planted. Magnesium sulphate may not have been applied in a quantity large enough or it may not have become sufficiently mixed through the soil to supply the amount of magnesium needed to completely overcome the chlorosis. It is a common observation that magnesium sulphate applied to soil persists in coming to the surface. Thus, not enough may have been available to the roots in all cases. The evidence for 1924, however, indicated that the magnesium sulphate performed a function similar to that of lime in tending to eliminate the chlorosis.

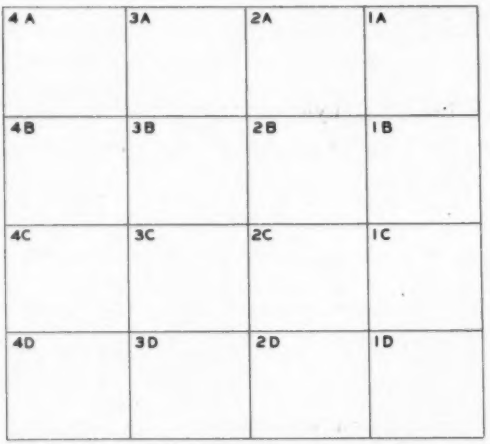

FIGURE 2.—Plan of North Corn Acre, the field used for the study of chlorosis; dimensions 214 by 203.5 feet; size of plots one-sixteenth acre. Sections A and B were limed in 1921 at the rate of 2 tons per acre; sections B and C had $MgSO_4$ applied at the rate of 200 pounds per acre in 1924 and 1927, and at the rate of 400 pounds per acre in 1928; section D received neither lime nor $MgSO_4$. The fertilizer treatment of plots 1, 2, 3, and 4 is shown in Table 1

TABLE 3.—*Percentage of normal and chlorotic corn hills in the various plots of North Corn Acre in 1924, 1927, and 1928*

Plot No.	1924				1927				1928			
	Normal hills	Chlorotic hills			Normal hills	Chlorotic hills			Normal hills	Chlorotic hills		
		Slightly	Medially	Extremely		Slightly	Medially	Extremely		Slightly	Medially	Extremely
1A	99.56	0.44	0	0	98.33	1.67	0	0	77.40	22.60	0	0
1B	99.52	.48	0	0	97.92	2.08	0	0	97.12	2.88	0	0
1C	80.86	19.14	0	0	84.85	15.42	0	0	97.12	2.88	0	0
1D	31.43	48.57	17.62	2.38	14.17	38.33	31.67	15.83	6.25	16.83	27.88	49.04
2A	98.66	1.34	0	0	92.92	7.08	0	0	75.38	21.61	3.01	0
2B	99.05	.95	0	0	97.08	2.92	0	0	98.50	1.50	0	0
2C	51.91	35.71	12.38	0	82.77	17.23	0	0	98.08	1.92	0	0
2D	11.49	22.01	45.45	21.05	2.97	12.29	26.27	58.47	5.80	16.91	29.95	47.34
3A	99.55	.45	0	0	94.12	5.88	0	0	82.21	16.83	.96	0
3B	98.55	1.45	0	0	96.25	3.75	0	0	94.23	5.77	0	0
3C	86.12	13.88	0	0	92.89	7.11	0	0	98.56	1.44	0	0
3D	17.62	50.48	30.47	1.43	32.08	58.33	9.17	.42	6.76	38.65	42.51	12.08
4A	96.64	3.36	0	0	97.91	2.09	0	0	96.15	3.85	0	0
4B	95.11	4.89	0	0	99.17	.83	0	0	96.64	3.36	0	0
4C	90.22	9.78	0	0	97.08	2.92	0	0	97.09	2.91	0	0
4D	78.12	20.54	1.34	0	30.12	54.81	14.23	.84	20.67	36.95	13.79	28.57

After the 1924 experiments corn was not again grown until 1927. In 1925 and 1926, when hay was grown, no magnesium was applied. The observations on chlorosis made in 1927 are shown in Table 3. The treatments with lime alone and with a combination of lime and magnesium sulphate were both effective in controlling chlorosis. On plots 3C and 4C the magnesium sulphate treatment was practically as effective as where it was applied in the limed area. On all the plots magnesium sulphate controlled chlorosis better than in 1924. This was evidenced by a greater percentage of normal hills and a less severe type of chlorosis. On the other hand, the chlorosis on section D, with the exception of that on plot 3D, was more prevalent in 1927 than in 1924 and was also more severe.

Corn was grown again in 1928, and the effects of the various treatments on chlorosis are shown in Table 3. It should be recalled at this point that 400 pounds of magnesium sulphate per acre were applied to sections B and C. Magnesium sulphate practically eliminated the chlorosis, a fact outstanding in the table. It was fully as effective when applied alone as when added to the limed area, and was more effective than lime alone. In 1928 the percentage of normal hills on section A was smaller than in 1924 and 1927. This may mean that the lime was no longer as effective in controlling the chlorosis as it was in the earlier years following its application. On section D, with the exception of plot 2D, where chlorosis was about as bad, there were fewer normal hills and a larger proportion of extremely chlorotic hills in 1928 than in 1927.

In 1928 two rows of tobacco were planted with the corn through each plot to check further the magnesium hypothesis. This was done because the writer was not familiar with the symptoms of magnesium hunger on corn but was somewhat acquainted with them on tobacco, and it was thought that the behavior of the tobacco, with reference to the cross treatments which had proved successful in controlling chlorosis of corn, might afford a means of distinguishing between the effects of the lime and magnesium sulphate.

On section D of every plot typical sand drown was noted on many of the tobacco plants within two weeks after they were set. It is unusual to find this type of chlorosis so early in the season, particularly when it is considered that the fertilizer used for the tobacco carried cottonseed meal in quantity equivalent to about 1,100 pounds per acre. Garner and his associates (*4*) had found in their experiments that about half this amount of cottonseed meal decidedly reduced sand drown. The tobacco on section D, however, showed a very severe case of sand drown, and by the end of the season scarcely a leaf on any of the plants in the entire section was free of it.

Sand drown of tobacco and chlorosis of corn both responded to the same treatments. On sections B and C, where the chlorosis was reduced to a minimum by the magnesuim sulphate treatment, the tobacco showed no evidence of sand drown. The line of demarcation between the plots on sections C and D could easily be distinguished by the absence of sand drown and chlorosis in the one case and their presence in the other. On section A there was evidence, similar to that for chlorosis on the corn, that the lime was not controlling the sand drown. Table 4 shows that 28.08 per cent of the tobacco plants were affected by sand drown.

If the records of Table 4 are compared with those of Table 3 it is evident that a correlation exists between the effect of magnesium sulphate on chlorosis of corn and on sand drown of tobacco. Since sand drown has already been proved to be due to a deficiency of magnesium, this correlation suggests very strongly that the lack of magnesia is the cause of chlorosis on the corn.

TABLE 4.—*Percentage of tobacco plants free of and affected by sand drown when planted on North Corn Acre in 1928*

Plot	Normal plants	Plants affected by sand drown		Plot	Normal plants	Plants affected by sand drown	
		Medially	Extremely			Medially	Extremely
1A	54.39	45.61	0	3A	63.16	36.84	0
B	100.00	0	0	B	100.00	0	0
C	100.00	0	0	C	100.00	0	0
D	3.39	8.48	88.13	D	0	12.73	87.27
2A	94.64	5.36	0	4A	75.47	24.53	0
B	100.00	0	0	B	100.00	0	0
C	100.00	0	0	C	100.00	0	0
D	11.86	37.29	50.85	D	0	12.73	87.27

Table 5 gives a summary showing the variation by years in the total percentage of chlorosis. The severity of the disease is disregarded here, but the figures accurately represent the proportion of the corn affected. On section D the tendency has been for the chlorosis to increase each year. On section C, where magnesium sulphate was added, the tendency has been just the reverse, the smallest percentage of chlorotic hills occurring the third year, 1928. Where both lime and magnesium sulphate were used (section B) chlorosis was controlled about equally each year. On section A, where lime alone was used, chlorosis was held in check in 1924 and 1927, but in 1928 there was evidence that the effectiveness of the lime was much reduced.

TABLE 5.—*Percentage of chlorotic hills of corn occurring in the various plots of North Corn Acre during different years*

Plot	Chlorotic hills occurring in—			Average	Plot	Chlorotic hills occurring in—			Average
	1924	1927	1928			1924	1927	1928	
1A	0.44	1.67	22.60	8.24	3A	.45	5.88	17.79	8.04
B	.48	2.08	2.88	1.81	B	1.45	3.75	5.77	3.66
C	19.14	15.42	2.88	12.48	C	13.88	7.11	1.44	7.48
D	68.57	85.83	93.75	82.72	D	82.38	67.92	93.24	81.18
2A	1.34	7.08	24.62	11.01	4A	3.36	2.09	3.85	3.10
B	.95	2.92	1.50	1.79	B	4.89	.83	3.36	3.03
C	45.09	17.23	1.92	22.41	C	9.78	2.92	2.91	5.20
D	88.51	97.03	94.20	93.25	D	21.88	69.88	79.31	57.02

These variations in chlorosis with the different seasons are interesting in connection with the precipitation records shown in Table 6. It has been a common observation that, when fertilization is the same, sand drown is more prevalent in seasons of excessive rainfall than in seasons of light rainfall. Table 6 shows that the rainfall for the growing seasons of 1927 and 1928 was about two and three times, re-

spectively, that of 1924. It also shows that about 27 per cent of the total rainfall occurred during the growing season of 1924, 35 per cent during that of 1927, and 59 per cent during that of 1928.

TABLE 6.—*Inches of rainfall during the growing season on North Corn Acre in 1924, 1927, and 1928*

Month	Rainfall in—		
	1924	1927	1928
May	2.21	4.83	3.25
June	1.28	3.37	6.97
July	1.75	3.40	6.23
August	3.11	5.01	8.40
Total	8.35	16.61	24.85
Total precipitation for the year	30.96	46.96	42.06

Chlorosis on section D, where no cross treatment of magnesium was made, increased each year. This increase is correlated with the increase in rainfall. In 1928, when the rainfall was heaviest, the chlorosis was most severe. Observations on tobacco grown in the Connecticut Valley showed practically no sand drown in 1924, somewhat more in 1927, while in 1928 it could be found in almost every field. Garner and his associates (*4*) also recognized that sand drown is more widespread in seasons of heavy rainfall. It therefore seems that a relation exists between the manner in which excessive rainfall during the growing season affects chlorosis and the manner in which it affects sand drown. This relation would seem to indicate that the chlorosis was caused by the same deficiency as sand drown.

EFFECT OF DIFFERENT FERTILIZERS ON THE DEVELOPMENT OF CHLOROSIS

Reference to Table 1 will show the annual fertilizer treatments received by the different plots from 1921 to 1928, inclusive. Prior to 1921 plots 1 and 3 had been duplicates, but since 1921 the high phosphoric acid treatment has been discontinued on plot 1. Until 1921 the only difference between the fertilizer treatment of plots 2 and 4 was that plot 4 received annually, from 1907 to 1918 inclusive, 400 pounds of basic slag per acre, but since 1921 the high potash has been omitted from the treatment of plot 4.

On section D, where there has been no cross treatment, it is possible to compare the influence of the fertilizers on the development of chlorosis. Table 7 shows the relative amounts of chlorosis in 1924, 1927, and 1928 by plots for section D. The outstanding fact revealed by this table is that chlorosis was present on all plots regardless of the fertilizer used. On plot 1D, where phosphorus was omitted, there was apparently less chlorosis in 1924 than on plot 3D, where the original high phosphorus treatments were continued, but in 1927 the reverse was true. In 1928 these two plots had about the same amount of chlorosis. Plot 2D, where the high potash treatment was continued has shown consistently a larger proportion of chlorotic hills than plot 4D, where the potash treatment was discontinued. In the data presented above it was shown that both magnesium and lime treatments were curatives for the chlorosis. The basic slag used on plot

4 for 12 years carried both lime and magnesium. This may account for the reduced amount of chlorosis on plot 4D as compared with that on other plots. So far as plots 1, 2, and 3 are concerned, there is not enough consistency in the data to show that the different fertilizers used have influenced the proportion of chlorotic hills. In 1928, when conditions were most favorable for chlorosis, these three plots were almost identical so far as the number of chlorotic hills was concerned.

Except in plot 4, where some magnesium and lime were added in the form of basic slag, it can be said that the development of chlorosis was independent of the fertilizers used. If, as suspected at the beginning of the experiment, chlorosis was due to aluminum toxicity the proportion of chlorotic hills should have been reduced by the high applications of phosphorus; but since the amount of chlorosis seemed to be about as large on the high phosphorus as on the other plots the aluminum hypothesis appears untenable.

TABLE 7.—*Influence of different fertilizer treatments on the chlorosis of corn in section D of North Corn Acre*

Plot	Chlorotic hills occurring in—			Average	Plot	Chlorotic hills occurring in—			Average
	1924	1927	1928			1924	1927	1928	
	Per cent	*Per cent*	*Percent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*	*Per cent*
1D	68.57	85.83	93.75	82.72	3D	82.38	67.92	93.24	81.18
2D	88.51	97.03	94.20	93.25	4D	21.88	69.88	79.31	57.02

EFFECT OF CHLOROSIS ON YIELD

In Table 8 are presented figures which show the effect of chlorosis on the yield of corn. In compiling the figures four plots were averaged in each case to obtain the yield per acre for the different sections. In 1928, however, because of wireworm injury on certain sections of the field, only the yield records for those plots where the injury was slight were included. Even with this precaution the yields indicate that the amount of replanting made necessary by the wireworm injury, particularly on sections B and C, should be considered. Except in 1928, sections A, B, and C are very close in yield of both corn and stover. Section D each year has consistently yielded less of both corn and stover than the other sections. Considering the average yield of corn for sections A, B, and C as compared with that for section D, they outyielded the latter by about 21 per cent in 1924, 6 per cent in 1927, and 34 per cent in 1928. If stover is considered similarly, the differences are 7 per cent for 1924, 4 per cent for 1927, and 16 per cent for 1928.

The average percentage of chlorotic hills for sections A, B, and C varied from 2.57 to 11.89. It should be recalled that section A received the lime treatment, section B the lime plus magnesium sulphate, section C the magnesium sulphate alone, and that section D was reserved as a check. It is evident from Table 8 that the chlorosis did not appreciably affect the yield of corn or stover when the chlorosis was controlled as it was on sections A, B, and C. On section D, however, where the average percentage of chlorotic hills reached

78.57 per cent, there was a depression in yield. The corn yields appeared to be more severely affected than the stover yields. These data suggest that chlorosis, though manifesting itself mostly on the foliage of the corn plant, does not affect the growth of the plant as much as might be expected. The major effect of chlorosis was on the production of seed.

TABLE 8.—*Effect of chlorosis on the yield per acre of grain and stover*

[Dry weight basis]

Section	Yield in—						Average		Chlorotic hills
	1924		1927		1928				
	Corn	Stover	Corn	Stover	Corn	Stover	Corn	Stover	
	Bushels	*Pounds*	*Bushels*	*Pounds*	*Bushels*	*Pounds*	*Bushels*	*Pounds*	*Per cent*
A	33.5	1,864	38.5	1,901	37.0	2,112	36.3	1,959	7.60
B	33.9	1,945	37.7	1,785	32.5	1,879	34.7	1,870	2.57
C	31.4	1,826	39.6	1,980	31.4	1,828	34.1	1,878	11.89
D	27.2	1,749	36.4	1,820	25.0	1,676	29.5	1,748	78.54

GREENHOUSE STUDIES

In the above discussion of field work it was evident that both lime and magnesium sulphate possessed the ability to control the chlorosis. In the greenhouse, an attempt was made to determine whether the effect of the lime was due to its ability to counteract aluminum toxicity or to the magnesium it contained. Soil was taken from the section of the field showing the highest percentage of chlorotic corn hills, placed in pots, and fertilized uniformly with a complete fertilizer free of magnesium. The fertilizer applications and the treatments shown in Table 9 were made on January 12 and the corn was planted on January 26. The soil was maintained at a uniform moisture content while the corn was growing. The corn was thinned to two stalks per pot. On April 18 the crop was harvested.

TABLE 9.—*Influence of different fertilizer treatments on the yield and extent of chlorosis of corn grown in the greenhouse*

Pot No.	Treatment per acre	Yield of dry matter per pot	Extent of chlorosis
		Grams	
1-4	400 pounds $MgSO_4 7H_2O$	40.48	None.
5-8	800 pounds $MgSO_4 7H_2O$	38.49	Do.
9-12	1,200 pounds $MgSO_4 7H_2O$	33.10	Do.
13-16	Check	29.15	Extreme.
17-20	2 tons agricultural lime [a]	30.15	Slight.
21-24	2 tons C. P. $CaCO_3$	23.83	Extreme.
25-28	1,000 pounds superphosphate	33.11	Do.
29-32	2,000 pounds superphosphate	32.49	Do.

[a] Contained 5 per cent MgO.

In Table 9 are recorded the yield of dry matter per pot and notes on the appearance of the corn with reference to chlorosis. Where magnesium sulphate at the rate of 400 and 800 pounds per acre was

used the yields were highest and the corn was free of chlorosis. Where magnesium sulphate at the rate of 1,200 pounds per acre was used the yield was not quite so good as where the smaller amounts were applied, but no chlorosis was noted. The smallest yields were obtained on the check pots and on those receiving the chemically pure calcium carbonate. The yield from the pots that received agricultural lime was very similar to that from the pots that received superphosphate. However, with only four replications the differences are in most cases too small to interpret with confidence.

The effect of the treatments on chlorosis were much more clear cut than on the yields. The first notable feature was that both magnesium sulphate and agricultural lime counteracted the chlorosis just as they did in the field. That a slight amount of chlorosis appeared

FIGURE 3.—The comparative effect of magnesium sulphate and superphosphate as preventives of chlorosis in corn grown on soil from fields which produced chlorotic corn: A, Magnesium sulphate was applied to the soil in this pot at the rate of 400 pounds to the acre; B, superphosphate was applied at the rate of 2 tons per acre

where the agricultural lime was used was attributed to the fact that the lime had had only about 14 days in which to become thoroughly mixed in the soil before the corn was planted. This was probably not long enough for the magnesium in the lime to become adequately available. Where the chemically pure lime (fig. 4) was used chlorosis was extreme. It was also extreme where the different amounts of superphosphate (fig. 3) were used. These results were interpreted as supporting the field observations and showing quite definitely that the ability of the lime to reduce chlorosis was due to the magnesium it contained, and not to any effect on aluminum toxicity. If the chlorosis were due to aluminum toxicity, the superphosphate and chemically pure lime treatments should have reduced the chlorosis just as much as did the agricultural lime. It would also be necessary to consider magnesium an antidote for aluminum poisoning, but for this there is no experimental support.

To check further the aluminum hypothesis, lettuce, an aluminum-sensitive crop, was grown in pots of two kinds of soil. The first of these soils produced extremely chlorotic corn in the field while the second produced corn practically free of chlorosis. In pots the corn behaved exactly as it did in the fields. The growth of lettuce on these two soils was similar and showed no evidence of aluminum injury. The average dry yield of lettuce per pot of three plants for the first soil was 8.158 gm. and for the second 7.509 gm., giving a difference of 0.649 gm. In view of the variations among the pots this difference was considered insignificant.

Another point bearing on the aluminum hypothesis was the fact that the soil on which the extremely chlorotic corn was produced has a pH value of 5.4. In view of the work of Burgess and Pember, such a soil reaction would hardly be expected to be associated with sufficient

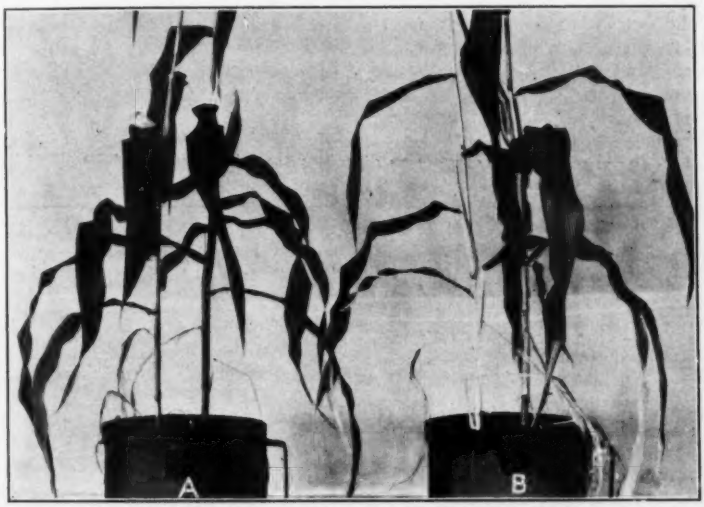

FIGURE 4.—The comparative effect of magnesium sulphate and chemically pure lime as preventives of chlorosis in corn grown on soil from fields which produced chlorotic corn: A, Magnesium sulphate was applied to the soil in this pot at the rate of 400 pounds per acre; B, chemically pure calcium carbonate was applied at the rate of 2 tons per acre

active aluminum to affect a plant so resistant as corn. The limed area of the field had a pH value of 6.1.

CHEMICAL STUDIES

When the chlorosis studies were first undertaken an attempt was made to obtain evidence on the aluminum hypothesis by chemical analysis of the plant. From the 1924 crop samples of corn showing slight, medial, and extreme chlorosis were taken. Iron and aluminum determinations were made on the roots and stalks. Hoffer (*6*) has called attention to the fact that when aluminum is available in the soil it will accumulate in the stalks of corn, particularly in the vascular plate tissue of the node. His results also indicated a relation between the aluminum content of stalks and injury due to corn root rots.

It was because of this work that the first chemical studies were confined to the stalk and roots. The removal of soil from the roots was not so easily accomplished as might have been desired. Although every precaution was taken to clean the roots, when the sample was ground there was evidence that a slight amount of soil was included.

In Table 10 are presented the results of the chemical analyses. The iron determinations on the stalks show no significant differences between the normal and the chlorotic plants. There appears, however, to be more iron in the roots than in the stalks, but this difference might possibly be accounted for by the small amount of soil unavoidably included in the sample. However, if this explanation is correct there should also be more aluminum in the roots than in the stalks, and such is not consistently the case.

The aluminum determinations showed higher amounts in the normal than in the chlorotic plants for both the stalks and the roots. This may be taken to indicate that accumulation of aluminum in the chlorotic plants was not responsible for the chlorosis. In addition, there was no evidence of any root rot associated with the chlorosis. According to Hoffer (*6*), the absence of root rot may be an indication that aluminum was not a factor.

TABLE 10.—*Influence of chlorosis on aluminum and iron content of corn stalk and roots*

Part of plants	Description of plants	Content of—	
		Fe_2O_3	Al_2O_3
		Per cent	*Per cent*
Stalks	Normal	0.013	0.193
	Slightly chlorotic	.010	.110
	Medially chlorotic	.013	.019
	Extremely chlorotic	.014	.029
Roots	Normal	.034	.171
	Slightly chlorotic	.029	.091
	Medially chlorotic	.022	.115
	Extremely chlorotic	.032	.136

In 1927 samples were taken again of normal and extremely chlorotic plants. The normal plants were taken from plot 1A, a plot practically free of chlorosis; the chlorotic plants from plot 2D, where chlorosis was most severe. An ash analysis was made.[3] In preparing the samples for analysis it was decided to exclude the roots because of the difficulty in getting a satisfactory sample and because it was evident that an analysis of the top part of the plant would be sufficient. The samples in each case were separated into nodes, internodes, leaf sheath, and leaf blade. This separation was made just after the plants had been dried.

The results of the analytical work are given in Table 11. In accord with the results obtained on the 1924 crop, no consistent differences in iron and aluminum content of the normal and chlorotic plants were apparent. In the nodal tissue, where the aluminum would be expected to accumulate, 0.06 per cent was found in the normal and 0.05 per cent in the chlorotic plants. Taking the plant as a whole,

[3] The writer is indebted to Dr. G. N. Hoffer, of the Indiana Agricultural Experiment Station, for his interest in this problem and for his assistance in having these analyses made in his laboratory.

the iron and aluminum content of the normal plants was very similar to that of the chlorotic plants.

The ash content of the leaf sheath and leaf blade was higher in the normal than in the chlorotic plants, while the reverse was true of the nodes and internodes. The average for the whole plant showed very little difference between the normal and chlorotic plants. The leaf sheath and leaf blade contained significantly more ash than the nodes and internodes.

TABLE 11.—*The effect of chlorosis on the mineral constituents of different parts of the corn plant*

Mineral	Percentage content of—								Average	
	Nodes		Internodes		Leaf sheath		Leaf blade			
	Normal	Chlorotic	Normal	Chlorotic	Normal	Chlorotic	Normal	Chlorotic	Normal	Chlorotic
Total ash	5.56	8.70	3.85	6.72	9.11	8.58	12.29	8.99	7.70	8.25
SiO_2	.46	.45	.78	.67	3.72	2.94	5.88	3.51	2.71	1.89
P_2O_5	.26	.20	.20	.16	.45	.40	.86	.81	.44	.39
Al_2O_3	.06	.05	.04	.06	.08	.11	.09	.06	.07	.07
Fe_2O_3	.01	.01	.01	.09	.07	.07	.07	.06	.04	.06
CaO	.99	.66	.47	.38	1.03	.74	1.67	.88	1.04	.66
MgO	.34	.10	.16	.06	.40	.12	.33	.11	.31	.10
K_2O	2.32	4.79	1.76	3.82	2.72	3.33	2.80	2.86	2.40	3.70

Silicon, phosphorus, and calcium were all found to be slightly more abundant in the normal than in the chlorotic plants. These elements were more plentiful in the leaf sheath and leaf blade than in the other parts of the plant. Plot 1A, where the sample of normal plants was taken, had received more phosphorus and calcium than plot 2D, where the sample of chlorotic plants was taken. This might explain the higher percentages of these two elements in the normal corn. Plot 2D had received more potash than plot 1A, and Table 11 shows consistently more potash in the chlorotic plants.

While the differences just mentioned were quite consistent they were not so large as those for magnesium. Table 11 shows on an average more than 300 per cent more magnesium oxide in the normal than in the chlorotic plants. This appeared to be the most significant result of the chemical analyses.

The field work showed that both lime and magnesium sulphate counteracted chlorosis; the greenhouse work showed that it was the magnesium in the lime which prevented chlorosis; and the chemical work now shows that the important chemical difference between the normal and chlorotic plants is in the magnesium.

DISCUSSION

The data presented show that the chlorosis on North Corn Acre was due to a lack of magnesium. The conditions which brought about the shortage of magnesium were: (1) The removal of magnesium from the soil by leaching and continuous cropping, and (2) the use of fertilizers practically free of magnesium for a long period.

Crops in general do not remove large quantities of magnesium from the soil. It has been estimated from the work of Lyon and Bizzell (*9*)

that corn may take between 15 and 20 pounds per acre. Jenkins (*7*) has estimated that a crop of tobacco removes in its leaves and stalks about 15 pounds per acre. According to these estimates the magnesium requirements of corn and tobacco are similar.

More important than the removal of magnesium by crops is the loss by leaching. According to the lysimeter experiments of Lyon and Bizzell (*9*), the magnesium lost by drainage may vary from 28 to 70 pounds per acre. On North Corn Acre the soil was much lighter than that with which Lyon and Bizzell obtained these results, indicating that even greater leaching may have taken place than was observed by these writers.

The belief that leaching has been a factor in lowering the level of magnesium on North Corn Acre is supported by the fact that chlorosis increased with increased rainfall. Garner and his associates (*4*) emphasized the fact that plants on light soils suffer most from sand drown. They also stated that excessive rainfall causes sand drown to become more widespread.

In view of the fact that the fertilizers used for a long period on North Corn Acre were practically free of magnesium, it was to be expected that the losses by drainage and crop removal should in time exhaust the soil. Because of its magnesium content, applications of farm manure might have prolonged the time before the appearance of chlorosis, but it is doubtful whether the manure, unless applied in very large amounts, would have maintained the magnesium supply indefinitely.

It has not been necessary up to the present for workers in the field of soil fertility to pay much attention to magnesium. They have been concerned chiefly with the commonly deficient nutrients, nitrogen, phosphorus, and potash. With continuous cropping, however, and the use of fertilizers practically free of magnesia, particularly on soils subject to considerable leaching, magnesium deficiency is likely to become more and more a problem.

As manure becomes less available, successful crop production must depend upon fertilizer chemicals. The introduction of higher analysis fertilizers means materials of greater purity. Thus the fertilizer practice of the future, unless attention is given to magnesium, is likely to assist in depleting the soil magnesium. In the fertilizer practice of the past magnesium has been contained in many of the low-grade materials as an impurity. However, it should be borne in mind that the soil conditions responsible for the magnesium deficiency here reported have developed with the use of the medium-analysis fertilizer materials commonly employed for the past 30 years.

Materials which may be used as sources of magnesium are lime, sulphate of potash-magnesia, vegetable organics, magnesium sulphate, and basic slag. The proper amount of these materials to use will depend on the amount of magnesium they carry and on how severely the crop may be suffering from lack of it. Garner and his associates (*4*) have estimated that about 20 pounds per acre of magnesium is sufficient to control sand drown. In the light of evidence secured by the writer this quantity may need modification to meet the variation in requirements induced by differences in rainfall and soil conditions. For example, during the season of 1928 sand drown was noted on tobacco on certain plots where approximately 30 pounds of magnesium

per acre were applied in the form of sulphate of potash-magnesia and cottonseed meal. The soil on which this observation was made was light and was situated on a slope, thus being subject to both leaching and erosion during a rainy season. For corn the experience on North Corn Acre indicated that between 20 and 40 pounds of magnesium per acre are sufficient to control chlorosis. Probably for average conditions applications of between 20 and 25 pounds per acre will give satisfactory results. In view of the amounts removed by drainage as reported by Lyon and Bizzell (*9*), it would hardly seem that the estimates above are adequate. Since the results in the field show them to be sufficient the indication is that some magnesium must be released by the soil.

SUMMARY

The work presented in this paper consisted in a study to determine the cause of chlorosis on corn growing in one of the experimental fields at the Massachusetts Agricultural Experiment Station. Two hypotheses were put forward to explain the chlorosis (1) aluminum toxicity and (2) lack of magnesia in the soil. Preliminary observations indicated that lime possessed the ability to control the chlorosis. Magnesium sulphate treatments were made for comparison with the lime treatments, and it was found that both treatments counteracted the chlorosis in the field. In the greenhouse, however, where magnesium sulphate was compared with chemically pure calcium carbonate, only the former was capable of controlling chlorosis. Antidotes for aluminum toxicity such as high applications of superphosphate had no influence on the chlorosis. Chemical analyses always revealed a larger amount of magnesium in the normally green than in the chlorotic plants. Aluminum, on the contrary, proved to be as abundant in the normal as in the chlorotic plants. Thus the conclusion was reached that lack of magnesia was responsible for the chlorosis.

Chlorosis of corn and sand drown of tobacco both became more injurious when heavy rainfalls occurred during the growing season. The yield of corn was found to be more affected by chlorosis than that of the stover, the latter being reduced only slightly.

The conditions that favor magnesia hunger are a light leachy soil, the use of chemical fertilizers free of magnesium and without manure, and continuous cropping. If the leachings from lysimeters are taken as a criterion, the removal of magnesia by drainage water is of greater consequence than its removal by crops.

In fertility programs of the future it may be necessary to consider magnesium more than in the past, especially for some of the lighter soil types. This seems particularly important in view of the fact that fertilizer mixtures are likely to be composed of purer chemicals.

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